Nucleic acid integrity and synthesis in relation to seed vigour in sugar beet

Thesis

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NUCLEIC ACID INTEGRITY AND SYNTHESIS IN RELATION TO SEED VIGOUR IN SUGAR BEET

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ABSTRACT

This research has been directed to understanding the nature of vigour in seeds of sugar beet and to methods for predicting germination success. The ratio of extractable RNA to DNA correlated significantly ($p<0.05$) with vigour assessments made using a range of germination tests and field trials when the seedlots from several cultivars were analysed together. However, comparisons of selected seedlots all of similar high quality produced few significant correlations between the laboratory assessments (RNA/DNA ratios and germination test measurements) and field performance.

Triploid cultivars yielded more extractable nucleic acids than diploid cultivars although overall the ratio of RNA to DNA was not affected by ploidy level. The amount of extractable RNA and DNA and the RNA/DNA ratio increased with fruit size although within the smaller size range used commercially, fruit size did not affect the RNA/DNA ratio.

A primed advancement treatment which improved field performance and produced faster germination at 9°C, had higher RNA/DNA ratios in comparison with thiram-steeped seedlots. The root tips from the embryos of advanced seeds also showed a higher percentage of nuclei with 4C DNA contents compared to those of untreated seeds but without cell division occurring indicating that the advancement treatment facilitated RNA synthesis and DNA replication. The RNA/DNA ratios of artificially aged seeds did not reflect the loss in germination seen in the standard germination test. Artificially ageing seeds produced a slightly lower percentage of root tip nuclei with a 4C DNA content although this decrease was more pronounced for advanced then artificially aged seeds.

At 24°C, seedlots differing in vigour showed equal capabilities for the repair of single strand DNA breaks following γ-irradiation, but had different capabilities for repair at 9°C. Limited repair occurred in the seedlot with poor performance under sub-optimal germination conditions, suggesting that the ability to repair DNA under stress could be a useful indicator of overall seed vigour.

ABBREVIATIONS

A_x	absorbance in optical units at wavelength, x nm
ANOVA	analysis of variance
A-T	adenine-thymine bases
ATP	adenosine triphosphate
ATPase	adenosine triphosphate synthetase
C	DNA content; 1C= unreplicated haploid chromosome complement
c.a.	approximately
cm	centimetre(s); 10^{-2} of a metre
CMS	cytoplasmic male sterility
cv.	cultivated variety (cultivar)
cvs.	cultivars
d	day(s)
dCMP	deoxycytidine monophosphate
DMP	diethylmercuric phosphate
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
ds DNA	double stranded DNA
dT	deoxythymidine
EB	extraction buffer
ed.	editor (used in references)
EDTA	ethylenediamine tetraacetic acid
estab%	number of seedlings counted at the 4-6 true leaf stage in the field
et al.	et alia
F1	first filial generation
g	according to context, gramme(s) or gravitational force
G%	percentage of seeds which have germinated
$G\%_{wet,dx}$	percentage of seeds which have germinated on day x of the wet stress test
$G\%_{20C,dx}$	percentage of seeds which have germinated on day x of the standard germination test at 20°C
h	hour(s)

H%	percentage of seeds which produced hypocotyls larger than 2 cm
$H\%_{wet,dx}$	percentage of seeds which produced hypocotyls larger than 2 cm on day x of the wet stress test
$H\%_{20C,dx}$	percentage of seeds which produced hypocotyls larger than 2 cm on day x of the standard germination test at 20°C
HCl	hydrochloric acid
H.M.W.	high molecular weight
I	intensity of fluorescence
kg	kilogramme(s)
kgN/ha	kilogramme(s) of nitrogen fertiliser per hectare
l	litre(s)
L.S.D.	least significant difference
M	molarity; the number of moles of solute per litre of a solution
mA	milliampere(s)
mc	moisture content
MET	mean time to emergence in the field
mg	milligramme(s); 10^{-3} of a gramme
MGT	mean time to germination in laboratory germination tests
ml	millilitre(s); 10^{-3} of a litre
mm	millimetre(s); 10^{-3} of a metre
mM	millimole(s); 10^{-3} of a mole
mRNA	messenger RNA
N	normality; the number of equivalents of solute per litre of solution
NaCl	sodium chloride
NaOH	sodium hydroxide
nm	nanometre(s); 10^{-9} of a metre
p	probability (associated with a numeral)
PEG	polyethylene glycol
poly(A)$^+$RNA	polyadenylated RNA species
pp.	pages (used in references)
PR	phenol:chloroform:isoamyl alcohol (25:24:1) reagent
r	Pearson's correlation coefficient
R	Spearman's rank correlation coefficient

RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rRNA	ribosomal RNA
RNase A	ribonuclease A
r.p.m.	revolutions per minute
s	second(s)
SDS	sodium dodecyl sulphate
S.E.	standard error
S.M.W.	small molecular weight
ss DNA	single stranded DNA
T_{30}	time to 30% of seeds sown germinating or emerging
T_{50}	time to 50% of seeds sown germinating or emerging
T_{50}*	time to 50% of the final count germinating or emerging
Tris	2-amino-2-(hydroxymethyl)-1,3-propanediol
tRNA	transfer RNA
UV	ultra-violet
V	volt(s)
Vis	visible
vol.	volume
vols.	volumes
v/v	volume for volume
w.h.c.	water holding capacity
w/v	weight for volume
µg	microgramme(s); 10^{-6} of a gramme
µl	microlitre(s); 10^{-6} of a litre
µm	micrometre(s); 10^{-6} of a metre
µmol	micromole(s); 10^{-6} of a mole
$\mu mol/m^2/s$	particle (photon) fluence rate (micromoles per metre squared per second)

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1.1. THE GENETICS OF SUGAR BEET

1.1.1. **The classification of sugar beet**

Beta vulgaris L is a member of the Chenopodiacae (Elliot and Weston 1993) and its classification is shown in Table 1.1. *B. vulgaris* has three subspecies, *B. vulgaris vulgaris*, *B. vulgaris maritima* and *B. vulgaris adanensis* (Letschert *et al.* 1994). The subspecies, *B. vulgaris vulgaris* contains six cultivated crops including sugar beet. *B. maritima* (wild maritime beet) was previously classified as a separate species to *B. vulgaris* but, due to the wild maritime beet and wild sugar beet being inter-fertile, it has become a subspecies of *B. vulgaris* (Clapham *et al.* 1987).

TABLE 1.1 The classification of sugar beet.

KINGDOM	Planta
PHYLUM	Spermatophyta (Angiospermae)
DIVISION	Anthophyta (Magnoliophyta)
CLASS	Dicotyledones
SUBCLASS	Archichlyamydeae
ORDER	Caryophyllales
FAMILY	Chenopodiaceae
GENUS	*Beta* L.
SPECIES	*Beta vulgaris*
SUBSPECIES	*Beta vulgaris vulgaris*

1.1.2. **The breeding of sugar beet**

The first synthetic cultivars available in Europe were diploids. These were the hybrids of lines which had been prepared by mass selection and progeny selection. A comprehensive description of the breeding of sugar beet has been written by Bosemark (1993) of which part of the following is a summary.

The normal diploid chromosome number of sugar beet is 18 (2n=2x=18) whereas in

autotetraploid sugar beet there are 36 chromosomes (2n=4x=36). Experiments on autotetraploid beets showed that they had a lower yield than the diploid but the triploid hybrid (produced by crossing the diploid with the tetraploid) produced the highest yield. Therefore triploid cultivars were developed in Europe for their greater productivity; the only problem being the production of 100% triploid seeds. The method of seed production was to allow diploid and tetraploid plants to pollinate each other freely resulting in 25% of seeds being diploid, 50% being triploid and 25% being tetraploid.

The 100% production of triploids was possible with the development of cytoplasmic male sterility (CMS) by Owen (1945) which prevented self-pollination on the CMS plants. At around the same time in the 1950s, monogerm (one seed per fruit) cultivars became available, 20 years after Savitsky and Bordonos first found a monogerm plant in Kiev. Multigerm seed clusters (produced by flowers developing in clusters) give rise to several seedlings which have to be expensively singled by hand in the field. Triploid monogerm forms, with only a single flower at each node of the inflorescence (Elliot and Weston 1993), are produced by crossing male sterile monogerm plants with multigerm pollinators. In most modern cultivars at least 90% of germinated seeds produce a single seedling (Durrant *et al.* 1986).

Since the introduction of CMS, most commercial cultivars available in Europe are monogerm triploid hybrids produced by a cross between a monogerm diploid female (CMS) and a tetraploid pollinator (see Table 1.2a). The pollinators originate from a heterogeneous population because the hybrids thus produced are more likely to show a stable performance over a range of environments than those produced from an inbred pollinator (Bosemark 1993). Triploid hybrids rather than diploid hybrids were bred because the monogerm female material, originating in America, was of low quality and it was thought that the unfavourable characters were more likely to be masked by a heterogeneous tetraploid (donating two copies of the genome) than a heterogeneous diploid pollinator (donating one copy of the genome).

Now that the monogerm female lines used have fewer weaknesses, selected diploid hybrids (see Table 1.2c) are commercially available with the added benefit of being easier to produce. Recently and increasingly in the future, diploid F1 cultivars (see Table 1.2d) will become commercially available in Europe. In this case, a highly selected inbred multigerm line, rather than a broad-based one, is used as the pollinator of a monogerm female. Using two inbred

lines allows a high degree of selectivity of characters but these lines need to be very vigorous to compensate for loss of genetic variation and they also need outstanding combining ability. The development of highly selected diploid F1 hybrids will be aided by new molecular techniques such as RFLP mapping and the identification of gene markers.

TABLE 1.2 Hybrid monogerm cultivars structures.

Cultivar	Status	Female(CMS)	Pollinator
a.triploid	many European commercial cultivars	inbred or F1 monogerm diploid	tetraploid from heterogeneous population
b.triploid	experimental	inbred or F1 monogerm tetraploid	diploid from heterogeneous population
c.diploid	many cultivars in USA	inbred or F1 monogerm diploid	diploid from heterogeneous population
d.diploid F1 cultivar	new commercial cultivars in Europe	diploid inbred monogerm	diploid inbred multigerm

Bosemark (1993) summarises the objectives of sugar-beet breeding programmes as follows:

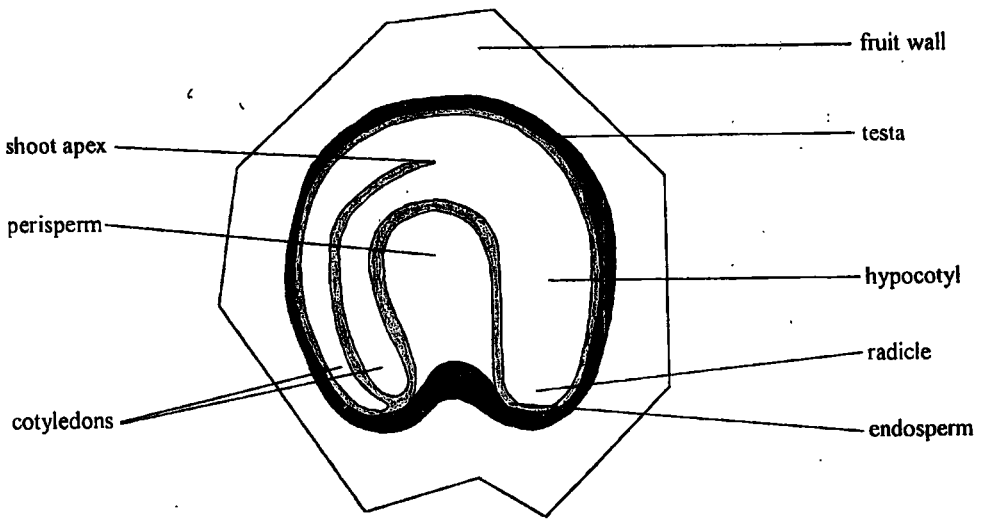
"to create stable, dependable varieties which give the highest possible yield of white sugar per unit area in relation to the cost of production and which meet various other requirements of the growers and the sugar factories".

Important characters which have been improved by breeding include root yield, sucrose content, impurities content and pest and disease resistance.

1.2. THE STRUCTURE AND COMPOSITION OF THE SEED

Following Richard *et al.* (1989), the term 'fruit' refers to the whole sugar-beet seed (including the pericarp) while the 'true seed' refers to the embryo plus its perisperm and testa (see Figure 1.1).

FIGURE 1.1 The structure of the sugar-beet fruit.



The sugar-beet true seed is composed of:

1) endosperm tissue (triploid cells derived from the fusion of the two polar nuclei of the ovule with one of the pollen nuclei at fertilisation);

2) cotyledons (embryonic reserves which form the first two leaves);

3) the radicle;

4) the perisperm (the nucellus which is residual parenchymal tissue from the mother plant).

The corky fruit wall (pericarp) and testa surround the seed. The true seed weight is about 20% of the fruit weight (Durrant 1980). True seeds can be isolated from the pericarp by prising off the operculum (otherwise known as the cap) with a mounted needle. The pericarp restricts the amount of water and oxygen that the true seed experiences; it also contains various germination inhibitors (Morris *et al.* 1984, see 1.4.2). The apical pore found in the cap may be an entry site for fungi, air and water (El-Nashaar and Bugbee 1981). The much larger basal pore is an important region for gaseous exchange (Santos and Pereira 1989).

In mature sugar-beet seeds, the major storage reserve is starch. This is predominantly located in the perisperm (Lawrence 1988, Lawrence *et al.* 1990) where only trace amounts of protein, soluble sugars and lipids have been found (Elamrani *et al.* 1992). Additional storage reserves in the embryo include protein (albumins, globulins and glutelins), soluble sugars eg. sucrose, lipids and only a trace of starch.

The sugar-beet seed differs from legumes in that starch is stored in the perisperm, the cells of which die during maturation drying, whereas in non-endospermic legumes, starch is stored in cotyledons which survive desiccation (Lawrence 1988). The situation in the seeds of grasses and cereals is the same as in sugar beet except that starch is stored in the endosperm not the perisperm. The perisperm in sugar-beet seeds is surrounded by a curled embryo. Lawrence *et al.* (1990) suggest that the cotyledons not only synthesise enzymes that degrade starch in the perisperm, eg. amylases, but that they also absorb these metabolites during seed germination and early seedling growth. Whereas lipids are the main respiratory substrate used during germination, starch and remaining lipids are only hydrolysed after root protrusion to sustain root and hypocotyl growth (Elamrani *et al.* 1992). A sugar-beet seed normally contains enough reserves to continue growth in the dark for six days after sowing before sugar starvation symptoms develop; for example, after twelve days, greening of the cotyledons is

incomplete on exposure to light (Elamrani *et al.* 1994).

1.3. THE DEVELOPMENT OF THE SUGAR-BEET SEEDLING

Seedling development may be divided into three stages: germination; emergence and establishment.

1.3.1. **Germination**

Bewley and Black (1978) define germination as:

"those processes which begin with water uptake and which successfully terminate with the emergence of the radicle or hypocotyl through the seed coverings".

Sugar-beet seeds can be said to have germinated once the root tip is clearly visible (Durrant 1980). Germination of the sugar-beet seed is epigeal. The radicle emerges first and then the cotyledons are carried above ground as the shoot grows. The cotyledons turn green to form leaves instead of remaining inside the seed, ie. the cotyledons are transformed into photosynthetic tissue upon exposure to light (Elamrani *et al.* 1994).

Sugar beet is a biennial plant but the storage root is harvested in the first year. If the root is not harvested and the plant experiences low temperatures of 5-10°C (Stout 1946) or air temperatures of less than 12°C (Durrant *et al.* 1993) together with long days, then in the second year the plant flowers. Sugar-beet flowers are mainly wind pollinated and sometimes insect pollinated (Free *et al.* 1975) to produce seeds. It is possible for the plant to show stem elongation (usually followed by flowering) even in the first year of growth if the seedling experiences sufficient low temperatures for vernalisation. This development is called bolting and produces sugar beet with lignified roots with a low sugar yield and if the inflorescences are not removed, then they may cause a weed beet problem for the next crop. The seedling becomes more sensitive to vernalising conditions as it develops (rev. Lexander 1980) although once leaves reach full expansion their sensitivity decreases (Crosthwaite and Jenkins 1993).

1.3.2. **Emergence**

Emergence of the sugar-beet seedling has occurred once it is visible above ground level (Durrant 1980). Normally it is the hypocotyl hook which appears first. Typically, commercial sowings are made at a soil depth of c.a. 2-3 cm.

1.3.3. **Establishment**

Seedlings are established once they have reached the 4-6 true leaf stage and are then likely to survive until final harvest (Durrant 1980).

1.4. FACTORS AFFECTING SEEDLING DEVELOPMENT

1.4.1. **The environment**

Lexander (1981) reviewed the physical and physiological seed characteristics influencing sugar-beet field emergence. Table 1.3 by Khan *et al.* (1979) summarises some of the environmental factors affecting seed performance in soil.

1.4.1.1. Temperature

Like other seeds, sugar-beet seed germination is affected by temperature. In general, sugar-beet seeds will not germinate at temperatures below 3°C and germination increases at higher temperatures. Above 42°C, the germination rate starts to decrease. The earlier sugar-beet seeds are sown and emerge in the field, the earlier maximum leaf area is achieved, more radiation is intercepted during the growing season and therefore the greater is the final yield (rev. Scott and Jaggard 1993). The problem with planting too early (apart from temperatures being too low for germination) is that seeds and seedlings may experience vernalising conditions which trigger bolting. Therefore in the U.K., farmers are recommended not to sow before 10 March.

TABLE 1.3 Environmental factors which affect seed performance (from Khan *et al.* 1979).

Type of factor	
physical	supra- and suboptimal temperatures unfavourable light conditions drought flooding unfavourable gaseous environment
mechanical	soil texture and composition depth of sowing crusting of soil surface compaction of soil and inadequate soil-seed contact
chemical	colloidal content salinity pesticides and herbicides and their residues toxic gases soil pH fertilizers
biotic	insects fungi and bacteria rodents and birds weeds

1.4.1.2. Water

The rehydration of seeds is the initial step towards germination (Bewley and Black 1978). Generally, seeds show a triphasic absorption of water. Initially water is absorbed quickly due to the dry seed having a lower water potential than the surrounding moist substrate (phase I). In phase II, water uptake slows down and the seed prepares to germinate. The mobilisation of stored reserves, eg. the hydrolysis of the starch in the perisperm, and elongation of the radicle occur in phase III along with a further and continuous increase in water uptake.

In sugar beet the pericarp controls the rate of water absorption which is partly due to the pericarp being composed of spongy tissue and salt crystals (Lexander 1981). Too much water may make the seed hypoxic (Richard *et al.* 1989) due to reduced oxygen diffusion, ie. when the pericarp is fully saturated with water or when a continuous water film is present around the fruit.

During the period when the seed germinates and immediately after, the moisture supply is critical (Durrant 1980). To germinate, sugar-beet seeds need to imbibe water equivalent to 26-30% of the air-dry seed weight (Durrant 1980) and further water is needed for seedlings to emerge. In addition, immature fruits absorb more water than ripe fruits (Snyder and Zielke 1973). Rate of germination is greatly dependent on rainfall (Durrant *et al.* 1988a). If field conditions are very wet during the first three days of sowing then emergence is reduced by up to 25%. Alternatively, rapid soil drying within the three-day period can give up to 35% fewer seedlings (Durrant 1980).

1.4.1.3. Other factors

The seedbed should have a fine moist tilth to permit good seed-to-soil contact (SBREC 1989) and prevent a physical barrier to radicle emergence. Delayed emergence may create sugar starvation in the seedling and prevent greening of the cotyledons (Elamrani *et al.* 1994).

Although the sugar-beet crop requires sodium to grow, the seeds show delayed germination in soils treated with sodium-containing fertilisers too close to the sowing date. Once the soil sodium concentration has been diluted by rain and irrigation, germination occurs (Durrant *et al.* 1974).

Emergence decreases if nitrogen levels in the soil are too high (Draycott 1972). Draycott *et al.* (1983) showed that broadcasting 125 kgN/ha slowed the rate of emergence and decreased the number of plants established but once the seedlings had emerged, the plants grew faster. Therefore, seeds should be sown with the addition of a small amount of fertiliser (40 kgN/ha) (Draycott *et al.* 1983) and more can be added once seedlings reach the 2-4 true leaf stage.

In addition to the above environmental factors, sugar-beet seedling establishment is reduced by pests eating the seeds, particularly mice, and by seed-borne fungal infections eg. *Phoma betae*, although pests and diseases rarely prevent viable seeds from germinating but rather affect growth once the radicle has emerged. Pests, particularly birds, tend to target seedlings which emerge first (Durrant *et al.* 1988a). Table 1.4 summarises the losses calculated at each stage in seedling development (Durrant 1988, Durrant *et al.* 1988a).

TABLE 1.4 Summary of losses (per 100 seed stations) during crop development.

Stage	% of plants completing each stage	Average losses (%)	Range of losses (%)	Reasons for loss
sowing		5[a] **2.8**[b] 4[c]	0-14[a] **1-5**[b] 1-8[c]	seeds drilled in wrong position
germination	92[a] **92**[b] 91[c]	8[a] **6**[b]	3-14[a] **3-11**[b]	empty, dead or damaged seeds
pre-emergence		16[a] **12.4**[b] 18[c]	2-42[a] **6-19**[b] 6-33[c]	non-optimal soil moisture, plant roots damaged by soil pests, disease, soil capping or stones
emergence	71.5[a] **79**[b] 71[c]			non-optimal soil moisture
post-emergence		6.15[a] **6.5**[b] 7[c]	0-16[a] **2-31**[b]	wind erosion, birds, soil, pests, herbicides, fungal diseases, grazing by rabbits and hares
establishment	61[a] **72.5**[b] 64[c]			

[a] Durrant 1988 (data from 1980-81; 125 crops)

[b] **Durrant *et al.* 1988a (data from 1978-81; two sowings/year)**

[c] Durrant 1980 (data from 1980; 71 crops)

1.4.2. **The seed coat**

The seed coat may retard germination by the ovary cap being too tight (Peto 1964, Nelson *et al.* 1984), by restricting water (Akeson *et al.* 1980) and oxygen or by the presence of inhibitors (Snyder 1959, Morris *et al.* 1984). Since hypocotyl growth in isolated true seeds increased earlier than in whole fruits, the pericarp appears to exert a retarding action on germination (Richard *et al.* 1989).

As early as 1939, Froeschel demonstrated that water leached through sugar-beet fruits would

inhibit seed germination in at least 29 other species (Morris *et al.* 1984). The seed coat composition has been analysed by a large number of workers to produce an extensive list of chemicals, in particular, phenolic compounds such as p-hydroxybenzoic acid (Battle and Whittington 1969) and oxalic acid (Synder *et al.* 1965). Explanations for the inhibitory properties of the chemicals range from the various phenolic compounds competing for oxygen (Coumans 1977) to the presence of the small inorganic ions creating a higher osmotic potential in the fluid (Junttila 1976, Morris *et al.* 1984). The advantage of pre-washing sugar-beet seeds to remove such chemicals before planting has been supported by several workers including Longden (1973) and Klitgard (1978) but disputed by Morris *et al.* (1984). The advantages of washing were also seen in the related red table beet (Khan *et al.* 1983). Morris *et al.* (1984) suggested that the lack of advantage found by them may be due to their use of rubbed seeds in comparison with the other workers who used raw seeds (although it was acknowledged that Longden had also used rubbed seeds).

A full review of the composition of the sugar-beet true fruit was compiled by Lexander in 1980. In the same review, Lexander examined the effect of the environment during seed production on the inhibitor contents. In summary, irrigation, large nitrate and potassium fertiliser applications, low temperatures (particularly during seed maturation) and early seed harvesting lead to increased inhibitor levels.

1.5. SUGAR-BEET SEED PRODUCTION AND PROCESSING

1.5.1. **Seed production**

Bornscheuer *et al.* (1993) has reviewed seed production methods. Most seeds for the European market are grown in the Mediterranean area because rain and low temperatures during seed ripening induce flowering. Also during flowering and seed production, higher temperatures are more optimal (15-20°C and greater than 12°C respectively). Low temperatures during seed maturation lead to seeds with lower germination percentages (Lexander 1980, Wood *et al.* 1980), increased inhibitor levels in the seed coat (Lexander 1980), tighter seed caps (Snyder and Hogaboam 1963), increased *P. betae* infection (Asher and Payne 1989), more bolting (Lexander 1980, Wood *et al.* 1980) and delayed ripening (Battle and Whittington 1969) leading to more underdeveloped seeds.

Wet conditions, through rain or irrigation, can delay maturation (Battle and Whittington 1969), lead to increased inhibitor levels in the seed coat (Lexander 1980) and favour *P. betae* infection (Leach and MacDonald 1976, Asher and Payne 1989).

1.5.2. **Seed harvesting**

Before harvesting the seeds, the pollinator plants are removed to ensure that only the fertilised seeds from the maternal line are harvested. Conditions need to be dry because if the seed moisture content is greater than 15%, then seed quality is lost during storage. The actual date of harvesting is a compromise between seed quality and seed yield. The later harvest ensures greater germination percentages because more seeds are mature but early harvesting reduces loss of seeds through shattering and bird damage (Austin and Longden 1968). However, Grimwade *et al.* (1987) found that if fruits were very mature when harvested then they had abnormally thick pericarps which restricted germination. As seeds mature, the fruit colour and moisture content changes but Snyder (1971) found these were not reliable indicators of when to harvest.

1.5.3. **Seed processing**

1.5.3.1. Method of analysis

Seedlots are subjected to a range of tests including germination tests and testing for the presence of specific seedborne diseases (Tonkin 1994). The seed moisture content and purity of the seedlot, in terms of sugar-beet seed weight in comparison with weed seeds and rubbish, are determined.

1.5.3.2. Rubbing

Rubbing sugar-beet seeds removes excess cortex (Durrant *et al.* 1986) which reduces the amount of inhibitory material (Santos and Pereira 1989). Rubbing also removes most of the sites of *P. betae* infection (Leach and MacDonald 1976) because more than 95% of *P. betae* infection is associated with the fruit wall (El-Nashaar and Bugbee 1981). In addition, rubbing creates seed bulks which are more uniform in size. Commercial bulks must be produced with

a seed size distribution that allows the seeds to be pelleted within a prescribed size range (see 1.5.3.5).

1.5.3.3. Grading

Seeds are separated into size fractions commercially by using slotted screens or gravity tables (Durrant and Mash 1990a). Experimentally, seeds are fractionated in air columns by density (Durrant and Loads 1990). Hogaboam (1961) and Longden *et al.* (1971) categorised fruits into sound, seedless, shrivelled, doubled or underdeveloped using X-ray analysis. Empty seeds are easier to separate by density because they are very light but it is difficult to separate bigerms from monogerms, or full but dead seeds. In general, larger seeds are more mature and are therefore more likely to germinate (Hogaboam and Snyder 1964, Wood *et al.* 1977, Milosevic *et al.* 1992). Larger seeds also give bigger responses to pretreatments (Scott *et al.* 1972) which may be explained by there being more underdeveloped seeds in lower size fractions which would not develop anyway.

1.5.3.4. Seed treatments

A review of sugar-beet seed treatments has been completed by Durrant *et al.* (1986). Seed treatments have been used in an attempt to increase rate of germination, synchronise emergence, increase stand establishment and control *P. betae* infection. Treatments that seemed to improve seed performance include soaking in salt solution (Lackey 1948, Durrant *et al.* 1983), growth regulator solutions (Scott *et al.* 1972, Akeson *et al.* 1981, Durrant and Mash 1991), fusicoccin solution (Nelson *et al.* 1984), dilute acid (Lackey 1948, Peto 1964, Akeson *et al.* 1980, 1981, Durrant and Mash 1991, Durrant *et al.* 1992), alkaline solution (Lackey 1948) and enzyme treatments eg. hemicellulase and pectinase (Peto 1964).

From 1962, the seedborne *P. betae*, which causes damping-off in young seedlings, was controlled by a diethyl mercuric phosphate (DMP) steep for 20 minutes (Durrant *et al.* 1988b) but in 1989, it was replaced by a 0.2% thiram (tetra-methyl thiuram disulphide) steep for 12 hours at 25°C (Durrant and Mash 1991). DMP was replaced by thiram because of the toxicity of mercury (Prince and Durrant 1990). Steeping seeds is more effective than field application methods (Halmer 1987) because it ensures an even distribution of fungicide between seeds and

a greater penetration of fungicide to control deep-seated infection (Durrant *et al.* 1986). Steeping sugar-beet seeds produces quicker emergence in the field (Prince and Durrant 1990) and 3% more established plants (Durrant and Mash 1992). In fact, steeping can also reduce seed coat inhibitor levels (Lexander 1981) and loosen the cap (Peto 1964).

There are also problems with using thiram; it may be phytotoxic (Gott *et al.* 1989) and it has already been banned in some countries, eg. Sweden, for environmental or safety reasons. The thiram steep for 12 hours at 25°C may result in seeds which produce more bolters (Durrant and Mash 1990b) and in some circumstances, the thiram steep can be reduced to 6-8 hours.

In addition to steeping seeds in fungicide, there are a number of seed pretreatments which have been tried on sugar beet including hardening/advancing, priming and fluid drilling.

1.5.3.4.1. *Hardening/advancing*

Hardening is a treatment where seeds are wetted and dried back (Heydecker 1974) which may be repeated more than once (Thomas 1981). Genkel in 1946 carried out presowing hardening of seeds as an aid against drought damage and he showed that hardening sugar-beet seeds produced a 10% increase in yield (Genkel *et al.* 1964). Emergence can be advanced compared with untreated seeds (Austin *et al.* 1969) particularly in soils showing a water deficit (Henckel 1964), low temperatures (Durrant and Jaggard 1988), high temperatures, ie. 38°C, and high salinity (Nelson *et al.* 1984). Improved seed performance has been explained on the basis that hardening allows 'after-ripening' as well as pregermination (Austin *et al.* 1969) and that the dehydration stage induces physiological reorganisation such as protoplasmic elasticity (Henckel 1964).

The term 'hardening' has been replaced by seed advancement (Longden 1971). After presoaking sugar-beet seeds followed by air-drying, Durrant *et al.* (1974) found faster emergence, a 70% increase in seedling fresh weights and an increase in the number of seeds that germinated.

A particular form of advancement treatment, the primed advancement treatment, has been developed by Durrant and co-workers (Durrant and Jaggard 1988, Durrant and Mash 1990b,

1992, Durrant *et al.* 1993) for sugar beet. It has been tested for commercial use (Thomas *et al.* 1993,1994) and is now commercially available to farmers in the U.K..

The primed advancement treatment involves steeping seeds in a 0.2% thiram suspension at 25°C for eight hours, drying back to c.a. 124% of the original weight, storing at 25°C for 88 hours and then air drying. Seeds have to be dried back to c.a. 124% of the original weight for the advancing period because wet seeds held at 25°C for more than 36 hours will germinate. The treated seeds show a number of advantages: they produce fewer bolting plants (Durrant and Jaggard 1988, Durrant *et al.* 1993), emergence is quicker (Durrant and Jaggard 1988, Durrant and Mash 1992) and the final level of emergence is increased under extended wet conditions (Durrant and Mash 1992). The increased emergence may be a consequence of the rapid emergence because seedling growth is then less likely to be prevented by soils slumping or capping and well-established plants can tolerate more pest and disease attack (Durrant and Mash 1992).

In the advancement treatment, the warm period (25°C) following imbibition leads to partial devernalisation (Durrant and Jaggard 1988). As early as 1946, Stout showed that vernalisation could be reversed in sugar-beet seeds by storage temperatures of between 11 and 26°C. Because there is less chance of advanced seeds bolting, they can be sown 7-10 days earlier under U.K. conditions (Durrant and Mash 1990b). Yield depends greatly on the length of the growing season (Goodman 1968) so that a combination of earlier and quicker emergence means more solar radiation is intercepted by the plants (Durrant *et al.* 1993).

1.5.3.4.2. *Priming*

Heydecker and Coolbear (1977) define priming as allowing seeds to commence metabolism associated with germination under conditions preventing radicle emergence by using inert osmotica, eg. PEG600, salt solutions or limited water supply or permitting imbibition at non-injurious supramaximal or subminimal germination temperatures. Often, the definition of priming (or osmoconditioning) is limited to only soaking seeds in PEG or salt solutions. However, Durrant (1980) defines priming as the process which should bring all seeds to the same physiological stage, close to germination, by controlling the amount of water that the seeds encounter. The end result of priming is to increase the synchrony and the rate of

germination and this has been demonstrated in celery (Salter and Darby 1976), soybeans (Knypl and Khan 1981), leek (Brocklehurst *et al.* 1984), carrot (Szafirowska *et al.* 1981) and sugar beet (Heydecker 1974). Rush (1992) found that primed sugar-beet seeds, as compared to raw seeds, had higher rates of emergence and gave better final stands in soil artificially infected with *Pythium spp.*. Commercial priming is restricted to certain crops, such as lettuce, since it is difficult to prime large seed volumes because of problems of viscosity and resistance to oxygen transport in large PEG volumes (Brocklehurst *et al.* 1987). Matric forces of solid carriers devoid of osmotic solutes, eg. vermiculite, can also be used to control the hydration of seeds (Khan *et al.* 1995). Terms used include moisturising, solid matrix priming and matriconditioning and this treatment has been shown to improve sugar-beet emergence by 10% in early field plantings (Khan *et al.* 1995).

1.5.3.4.3. *Fluid drilling*

Allowing seeds to germinate in ideal conditions before sowing them in a gel is termed fluid drilling. Pre-germinated seeds have shown earlier and increased emergence in red (table) beet (Darby 1980) and sugar beet (Khan *et al.* 1995) although Gray (1981, 1994) has found that the improved performance is not consistent in sugar beet. This seed treatment is still at an experimental stage.

1.5.3.5. *Pelleting and film-coating*

In Europe most monogerm seeds have been pelleted since the mid-1960s (Halmer 1987). A powdered blend of coating material along with water is built up in layers around the seed and then dried (Halmer 1987). The aim is to achieve a larger (typically a two to three-fold increase in weight) and more regular shape to allow precision drilling (Longden 1975). The pellet is a good carrier for fungicides (Thompson and Burns 1989), insecticides, nutrients and plant growth regulators (Longden 1990), for example, insulating the seed from intimate contact with possible phytotoxic chemicals (Heydecker and Coolbear 1977). In addition, pelleting protects the seed cap from dropping off and hence the possible loss of the true seed, particularly when seeds have undergone an advancement treatment (Longden 1971).

In the past, pelleting led to a decrease in sugar-beet establishment (Vanstallen 1971) which

may have been due to the pellet reducing oxygen diffusion in wet conditions and reducing water uptake when conditions were dry. This was particularly true before 1984 in the U.K. because the pellet was based on a heavy clay whereas now it is a lighter-weight organic pellet (Prince and Durrant 1990).

Film-coating involves spraying a solution onto the outside of a seed or pellet. The advantages of film-coating pelleted seeds include an even application of pesticide in a controlled manner and a reduction in possible phytotoxic effects of pesticides to the seeds.

1.6. SEED ASSESSMENT

Seed quality and its assessment have been comprehensively reviewed and discussed (Delouche and Caldwell 1960, Woodstock 1973, McDonald 1975, Matthews 1981, Halmer and Bewley 1984, Hampton and Coolbear 1990, McDonald 1994). Seed assessment provides the grower with an estimate of the value of the seed (Perry 1981), allows breeders to select for certain characteristics and permits quality control following seed treatments. The term 'viability' describes whether or not a seed can germinate. The viability of a seedlot is therefore assessed under optimal conditions using the standard germination test for the species in question (ISTA 1976). The average viability of commercial sugar-beet seed bulks in the U.K. is c.a. 92% (Durrant *et al.* 1985a) and much higher than the 80% minimum germination of certified monogerm seeds (HMSO 1993). In addition to quantifying the viability of a seed bulk, it is also important for the seed companies and ultimately the farmer to know firstly, how well the seeds will germinate under non-optimal conditions in the field and secondly, how well a seed will perform once it has germinated. The term 'vigour' includes all those seed properties which determine the level of activity and performance during germination and seedling emergence (Perry 1978). The level of activity of each seed during germination is determined by biological processes such as enzyme reactions. Performance includes the rate of germination and emergence (Maguire 1962); for example, seeds which have been stored for a long period of time take longer to germinate (Ellis and Roberts 1980) which is one manifestation of low vigour. Germination and emergence in the field need to be not only fast to maximise light interception, but also uniform when harvesting is a once-over operation (Hampton and Coolbear 1990). Conditions for germination in the field are rarely optimal (Clarke and James 1991) so that a seedlot with high vigour is better able to germinate and emerge to produce a

normal, healthy seedling population under a wide range of conditions. Emergence is a critical stage in seedling development. Durrant *et al.* (1988a) found that it was more common for sugar-beet seedlings to die after germination but before emergence than at any other stage between sowing and establishment (see Table 1.4).

Vigour is a more sensitive indicator of the health of a seedlot compared to its viability (McDonald 1975) because vigour starts to decline before viability (Perry 1980) or may decline more rapidly than viability (Delouche and Caldwell 1960). A range of factors influence the vigour of a seed. Perry (1981) lists them as: the genetic constitution; the environment and nutrition of the mother plant; the stage of maturity at harvest; seed size, weight or specific gravity; the mechanical integrity; deterioration and ageing and pathogens. The factors which affect seed vigour are described in more detail as follows:

- Seed companies are able to maximise the vigour of the commercial seeds available by selecting for specific traits in breeding programmes and growing and harvesting seeds under the most optimal conditions (reviewed for sugar beet by Lexander 1981).
- Larger seeds of sugar beet are more likely to germinate (Milosevic *et al.* 1992) or emerge (Snyder and Filban 1970, Akeson 1981) and give bigger responses to pretreatments (Scott *et al.* 1972) so that larger seeds are selected during seed processing.
- Mechanical damage inflicted at harvest or during processing may impair vigour and accelerate deterioration (Perry 1980).
- The ageing and deterioration of seeds reduces vigour with the final stage being loss of germinability (Gibson 1979) ie. viability. To prevent loss of seed quality in this way, seeds need to be stored at a low temperature and moisture content (rev. Roberts 1972) although if the seeds are over-dried this may increase the rate of deterioration (Priestley 1986, Vertucci and Roos 1991).
- Seed-borne pathogens such as *Phoma betae* may kill the seed or seedling or reduce seedling emergence (Perry 1980) particularly those low in vigour which are more susceptible to microbial attack (Delouche and Caldwell 1960).

The performance of the seeds and seedlings can be artificially improved by applying pesticides and fungicides and carrying out seed treatments, such as priming, to accelerate emergence.

Does this mean that seed treatments could be said to improve the vigour of a seed or seedlot? Vigour is a multi-dimensional property of the seed (Hampton and Coolbear 1990) so that to show an improvement in vigour, the seed would not only need an increased germination rate but to also show other features of high vigour such as stress tolerance and prolonged storage ability (Matthews 1981). For example, Argerich and Bradford (1989) found that primed tomato seeds had faster germination rates and earlier seedling emergence but that uniformity of germination had decreased. Therefore, seed vigour is not improved by seed treatments, such as priming, because although certain criteria of vigour, such as increased germination rates, are satisfied, the other criteria may be not. Practically, seedlots following seed treatments are assessed in laboratory tests and so in general only a limited number of criteria are tested. In this situation, even if the other characteristics such as storage ability are not tested, then improved germination performance can be said to represent an increase in seed vigour.

Seed vigour is assessed in field trials or estimated in the laboratory. The performance of seeds in the field is quantified in a number of ways; for example, measuring the rate of emergence of seedlings, the proportion of seeds which reach the 4-6 true leaf stage at establishment, using a vigour index based on the appearance of the seedlings or by weighing seedlings. During the field trial the seed experiences a range of conditions which are not controllable and therefore a seedlot may perform as well as others under certain conditions and be given a high vigour score but if conditions are different the seedlot may perform less well than the other lots and be rated with lower vigour. Therefore, new cultivars of commercial sugar-beet seeds in the U.K. are assessed in field trials on different sites for a minimum of three years before they appear on the recommended list for farmers (SBREC 1989). In general, field trials are restricted to certain times of the year and demand a large input of time and resources, so that laboratory tests have been developed to estimate seed vigour and to test performance under selected stresses.

A vigour test is used to predict some aspect of seed performance, particularly, seedling growth rate, seedling emergence in the field, plant uniformity, crop yield and seed storability (Steiner *et al.* 1989). A vigour test becomes necessary once the standard germination test does not differentiate between seedlots which perform differently in the field (Matthews 1981). Generally the aim of a vigour test is to rank the seedlots in the same order as their performance in the field. The correlation between the vigour test results and the field

performance of the seedlots should be closer than the correlation between the standard germination test and the field performance (Perry 1981). There are a range of germination tests which are chosen to mimic certain field conditions (Woodstock 1973), eg. the wet stress test, but it is unlikely that any one test will be appropriate for a single species under all conditions (Hampton and Coolbear 1990). Other types of laboratory vigour assessments include biochemical tests which try to measure reactions or chemicals which are involved in seed cellular activity and physical tests which measure seed characteristics such as seed size and the structure of the seed by, for example, X-ray analysis (Longden *et al.* 1971). Results from a range of vigour tests have been combined to formulate an equation to predict seedling emergence (Steiner *et al.* 1989, Kim *et al.* 1994).

1.6.1. **Germination tests**

The standard germination test measures the viability of a seedlot under the controlled conditions of a measured substrate moisture content, an optimal temperature and using an inert medium of filter paper. A seed has germinated once the radicle is visible but ISTA (1981) recommends continuing the test in order to judge whether the seedling produced is normal, and then excluding all those seedlings which are imperfect from the final germination percentage. The standard germination test has been found to be a reliable test for predicting field emergence in sugar beet (Bekendam *et al.* 1987, Kraak *et al.* 1984) and onion (Kraak *et al.* 1984). Additional measurements, such as hypocotyl diameter (review Gibson 1979), counting hypocotyls at least 2 cm tall (Durrant and Gummerson 1990) in sugar beet and measuring root growth in tomato (Argerich and Bradford 1989), have also been developed as predictors of field performance.

The optimal conditions provided in the germination test rarely, if ever, occur in the field (Perry 1981) therefore stress germination tests have been developed. Examples for sugar beet include, low temperatures (Lovato and Cagalli 1992), wet conditions (Lovato and Cagalli 1992) and physical constraints such as the packed sand test (Akeson and Widner 1980). Some tests include a combination of potential stresses, for example, the cold sand test (Lovato and Cagalli 1992) where the sand is a physical barrier against germination. Another type of germination test includes exposing the seeds to a stress first such as controlled deterioration at a high temperature and moisture level (Powell and Matthews 1981, Burgass and Powell

1984), storing the seeds in moist cold soil for a week (Gibson 1979) or exposing the seeds to ionizing irradiation (Sheppard *et al.* 1989) and then germinating the seeds under standard conditions. For all these tests the final germination percentage indicates the tolerance of these seedlots to certain stresses but daily counting generates additional data in order to calculate the rate of germination under these stressful conditions.

1.6.2. **Biochemical tests**

Stress tests are time-consuming and shed little light on the underlying cause of seed performance so that much work has centred around developing a quick biochemical test which will quantify seed quality. One test still in use as a viability test utilizes the reduction of triphenyl tetrazolium chloride (Perry 1981) by the active mitochondrial dehydrogenase found in healthy tissue to produce a red colour change. A vigour test has been developed based on quantifying this colour change (Perl *et al.* 1978, Perry 1981, Perl and Kretschmer 1988) but problems include the changing permeability of deteriorating cells to the tetrazolium salt (Powell and Matthews 1977, Priestley 1986) and the strong subjective element to the assessment.

When seeds are imbibed, the rapid uptake of water causes physical disruption of the membranes (Simon 1984) producing the leakage of sugars (Coolbear *et al.* 1984, Lee *et al.* 1995), amino acids (Perl *et al.* 1978, Coolbear *et* al. 1984), electrolytes (Perl *et al.* 1978, Coolbear *et al.* 1984), nucleic acids, nucleotides and phenols (Deswal and Sheoran 1993) and sinapine (Hill *et al.* 1988, Lee and Taylor 1995). The extent of leakage depends on the mineral ion content of the cells, the ability to repair and re-organise cell membranes and natural differences or damage to the seed coat (Hampton and Coolbear 1990). The conductivity of seed leachate has been negatively correlated with field emergence particularly for legumes, eg. pea (Matthews and Whitbread 1968, Perry 1970) and cereals (Sheppard *et al.* 1989). The negative correlation has been explained as low vigour or non-germinating seeds not retaining their cell contents as effectively as seeds capable of germinating. This would indicate that during imbibition a low vigour seed would lose essential cell constituents which in turn, may attract micro-organisms (Woodstock 1973). The germination percentage of sugar beet in the standard germination test was negatively correlated with leachate conductivity (Durrant and Gummerson 1990) although other workers (Gibson 1979, Kraak *et al.* 1984, Bekendam *et al.*

1987) found the correlation to be unreliable because adhering floral tissue releases leachate (Gibson 1979).

Other biochemical measurements which have been suggested as methods to quantify the seed quality of different species include: measuring enzyme activity (Perl *et al.* 1978) such as glutamic acid decarboxylase (James 1968, Perl *et al.* 1978, Ram and Wiesner 1988), amylase levels (Lawrence 1988, Nandi *et al.* 1995) and ATPase (Sánchez-Nieto *et al.* 1992); rate of protein synthesis (Roberts and Osborne 1973, Reuzeau *et al.* 1992) and ATP levels (Ching 1973, Lunn and Madsen 1981) and synthesis (Perl and Kretschmer 1988). Respiration has been measured by oxygen uptake in wheat (Van de Venter and Grabe 1989) and the evolution of carbon dioxide in soybean (Abdul-Baki and Anderson 1973). Nucleic acid levels and synthesis have been quantified in seeds to give estimates of seed quality. For example, the rate of RNA synthesis has been related to the vigour of wheat seedlots during the early hours of germination (Blowers *et al.* 1980). Standard *et al.* (1983) related nucleotide levels in wheat embryos to high and low vigour or viability seedlots. Clarke and James (1991) found that the ratio of extractable RNA to DNA in leek embryos decreased with ageing and increased with priming. This agrees with work by Coolbear and coworkers (Coolbear and Grierson 1979, Coolbear *et al.* 1980) who found that there is a large increase in RNA, particularly rRNA, relative to DNA during priming. The relationship of seed quality and treatments with nucleic acids is discussed in greater detail in section 1.7.

1.6.3. **Aims**

The aim of part of the work reported in this thesis was to apply the RNA/DNA ratio concept to sugar-beet seeds to investigate whether the results would reflect the treatment that the seed had experienced and to develop this into a vigour test for assessing seedlots. Matthews (1981) recommends following a number of guidelines when a vigour test is being developed so that the RNA/DNA ratios of seedlots were related to assessments of seed performance made in the field and in a range of laboratory germination tests. The laboratory germination test results were also related to field performance to give an indication of when the vigour test was a better indicator of field emergence than the laboratory germination tests. Matthews (1981) also recommends only using seedlots which have more than the critical minimum germination percentage so that seedlots from commercially available cultivars were used. The effect of seed

characteristics such as fruit size on the RNA/DNA ratio were also investigated.

1.7. NUCLEIC ACID SYNTHESIS AND INTEGRITY IN RELATION TO SEED QUALITY

1.7.1. **Nucleic acid synthesis**

1.7.1.1. Nucleic acid synthesis during germination

In general, seed germination and seedling growth are associated with changes in enzymatic activities, respiration and the synthesis of protein, RNA and DNA although germination will occur without DNA replicative synthesis or cell division taking place (Bewley and Black 1978). Changes in the nucleus during early germination were reviewed by Deltour (1985). Protein synthesis will also be discussed here because it is dependent on nucleic acid levels and their condition. Hallam *et al.* (1972) described the first few stages of imbibition in rye. First the seed took up water and then after an hour, the respiration rate and the number of mitichondria and cristae had increased and by six hours, the seed was incorporating ^{14}C-uridine and thymidine-2-^{14}C into nucleic acids. RNA synthesis has been demonstrated following an hour of imbibition in bean (Walbot 1972) and in rye (Sen *et al.* 1975). DNA replication and cell division occur later in imbibition (Osborne *et al.* 1977). An increase in the 4C DNA content of embryo root tips following four days of imbibition in water has been demonstrated in the seeds of tomato (Bino *et al.* 1992) and pepper (Lanteri *et al.* 1993), indicating doubling of the DNA content by replication.

Which nucleic acids are essential for germination to occur? Cordycepin, which inhibits the polyadenylation of mRNA, also inhibits the germination of lettuce seeds which would indicate that post-transcriptional mRNA adenylation is essential for seed germination (Tao and Khan 1976). In addition Hecker and Bernhardt (1976) found that seeds imbibed in inhibitors of protein synthesis also failed to germinate which would indicate the importance of protein synthesis in the early stages of germination.

One interesting question which has been studied is whether early protein synthesis uses stored or *de novo* mRNA? The level of mRNA in a seed is determined by the rate of degradation of

stored mRNA and the synthesis of new mRNA during germination (Rushton and Bray 1987, Thompson *et al.* 1992). Potential mRNA molecules have long terminal sequences enriched in adenosine (A) residues which has made it possible to isolate mRNA by affinity chromatography on oligo-deoxythymidine (dT) cellulose (Simon 1984). Measuring poly (A)-rich RNA (poly(A)$^+$RNA), using an oligo dT-cellulose column (Payne 1977), indicated that the synthesis of this RNA species occurred at all stages of imbibition and germination. Bray and Smith (1985) showed that by inhibiting the synthesis of new poly(A)$^+$RNA with α-amanitin, germinating wheat embryos could degrade up to 60-70% of stored poly(A)$^+$RNA before there was any significant reduction in the rate of *in vivo* protein biosynthesis. However, Bewley and Black (1982) have questioned whether the use of such inhibitors can completely inhibit RNA synthesis in seeds. This amount of degradation is equivalent to the first two hours of germination at 20°C (Rushton and Bray 1987). In pea seeds, the rate of protein synthesis did not decline until 70-75% of poly(A)$^+$RNA had disappeared (Greenway *et al.* 1986). This deleted RNA may code for polypeptides that are important during seed maturation but less relevant during germination (Priestley 1986). Low poly(A) polymerase activity, which produces shorter poly(A) tails and may reduce the functional activity of mRNA, has been associated with decreasing viability in wheat embryos (Grilli *et al.* 1995). On these grounds, the polyadenylation of RNA following transcription has been proposed as being essential for the maturation of mRNA in wheat embryos. Factors affecting mRNA stability in higher plants have been reviewed by Green (1993).

1.7.1.2. Nucleic acid synthesis during seed treatments

Seed treatments, such as osmoconditioning, allow nucleic acid synthesis in seeds during the first stages of imbibition to be studied and the importance of nucleic acid accumulation for germination to be determined. During priming there is an increase in the pool size of nucleotides and nucleotide sugars (Bray *et al.* 1989). Also, during osmoconditioning in a PEG solution, there is an increase in the accumulation of RNA and protein synthesised in lettuce (Khan *et al.* 1978), tomato (Coolbear and Grierson 1979, Coolbear *et al.* 1980), peanut (Fu *et al.* 1988) and leek (Bray *et al.* 1989, Davison *et al.* 1991). RNA and protein synthesis began earlier, in comparison with untreated seeds, once the seeds were imbibed in water. This has been shown in lettuce (Khan *et al.* 1978), leek (Davison *et al.* 1991) and maize (Cruz Garcia *et al.* 1995). Coolbear *et al.* (1980) showed that treated seeds germinated more quickly and

accumulated RNA, so is the accumulation of RNA the process which produces a better seed performance or is it a reflection of a general enhancement of anabolic activity? Khan *et al.* (1980/81) found that even if RNA synthesis was inhibited during a seed treatment, on subsequent imbibition, radicle protrusion was not inhibited.

Low levels of DNA synthesis during pre-sowing treatments have been recorded in leek (Bray *et al.* 1989, Ashraf and Bray 1993) and tomato seeds (Coolbear *et al.* 1990). This DNA synthesis occurs without cell division (Bray *et al.* 1989) so that there is an increase of cells in osmoconditioned seeds with a DNA content of 4C (Bino *et al.* 1992, Lanteri *et al.* 1993). DNA synthesis and cell division occur earlier in osmoconditioned seeds once seeds are imbibed in water (Dell'Aquila and Taranto 1986, Cruz Garcia *et al.* 1995). For example, the onset of S-phase DNA synthesis began after 20 hours of imbibition in pretreated tomato seeds in comparison with 40 hours in untreated seeds (Coolbear and Grierson 1979).

The extent of nucleic acid accumulation during seed treatments is dependent on the concentration of PEG solution and the length of treatment before drying the seeds back to their original moisture content. Lanteri *et al.* (1994), using flow cytometry, found that PEG solutions which were less concentrated produced a greater proportion of cells with 4C DNA contents and decreased the mean time to germination (MGT) most efficiently. If low vigour rye seeds are imbibed in water for three to six hours and dried back to their original weight (as opposed to the much longer time period when using PEG solutions), then germination is improved and the rate of protein and DNA synthesis is enhanced on germination (Sen and Osborne 1974). These results indicate that the induction of DNA synthesis is regulated by the water potential of the surrounding environment. Longer osmoconditioning treatments may increase the susceptibility of seeds to deterioration (Deltour and Jacqmard 1974, Baker and Bradford 1995) which may be explained by the increased number of G_2 cells, ie. cells with a 4C content, being more sensitive to stresses such as radiation (Clowes 1965, Deltour 1985). Davison *et al.* (1991) showed that if leek seeds were osmoprimed for too long before drying back then not only was germination poor on rehydration, but also the rate of protein synthesis was decreased, there was more rRNA degradation and RNA levels did not increase. This has also been demonstrated in maize following 72 hours of hydration and then drying back, where upon rehydration, no RNA and DNA synthesis occurred (Crèvecoeur *et al.* 1988). Saracco *et al.* (1995) compared two priming treatments in pepper seeds. Both treatments improved

seed performance (ie. decreased MGT) but the seeds, in which DNA replication was induced by priming, were more sensitive to controlled deterioration. The priming treatment which did not induce DNA synthesis was based on using a high concentration of PEG solution combined with a long priming period. This indicates that the seed moisture content needs to be at a higher level during priming in order to facilitate DNA synthesis. This explanation is supported by Khan *et al.* (1980/81) who were not able to detect any change in the DNA content during an osmotic treatment of tomato seeds; this may have been due to the seeds not receiving enough water to permit DNA synthesis, or that the detection method was not sensitive enough.

1.7.1.3. Factors affecting nucleic acid synthesis in seeds

In addition to seed treatments, other factors such as, seed vigour and viability, the stage of maturity at harvest and storage conditions affect nucleic acid levels in seeds. Leek seeds with a high vigour level showed a higher incorporation of ^{14}C- amino acids into proteins and ^{3}H-thymidine into DNA during early germination in comparison with low vigour seeds (Bray *et al.* 1989, Meng and Li 1992). Low vigour embryos synthesise lower levels of RNA in the first hours of germination in comparison with high vigour embryos (Osborne *et al.* 1977, Blowers *et al.* 1980, Meng and Li 1992). More specifically, levels of mRNA ie. poly $(A)^{+}$-RNA during early germination were related to wheat seed vigour (Blowers *et al.* 1980). Rushton and Bray (1987) found that high vigour wheat embryos degraded stored mRNA and synthesised new mRNA more quickly during the first 12 hours of germination than medium and low vigour embryos. Low vigour embryos appear more sensitive to sub-optimal conditions. For example, Smith and Bray (1982) found that the difference in the amount of poly$(A)^{+}$-RNA synthesised for high and medium vigour seeds was much bigger during imbibition at 10°C than at 20°C. This was also the case when rates of protein and RNA synthesis (Blowers *et al.* 1980) and levels of nucleotides and nucleotide sugars in high vigour and low vigour embryos were compared (Standard *et al.* 1983). Low vigour embryos had much lower levels of nucleotides and nucleotide sugars at 10°C than high vigour embryos. The level of DNA precursors is important because pool sizes of nucleotides have been shown to affect nascent replicon maturation which is the process by which short pieces of newly synthesised DNA are joined together to form longer stretches (Schwartzman *et al.* 1984).

Loss of viability in seeds is accompanied by a significant loss of RNA (Bray and Dasgupta 1976, Ghosh and Chaudhuri 1984) and protein synthesising activity (Sen and Osborne 1977). Loss of viability of rice seeds has been shown to be accompanied by a decrease in poly(A)$^+$RNA (Ghosh and Chauduri 1984). Non-viable seeds showed a failure of nucleic acid and protein synthesis in rye (Hallam *et al.* 1973) and soybean (Meng and Li 1992). This was also accompanied by a decrease in the integrity of DNA and RNA even though total DNA, RNA and protein levels remained unchanged (Hallam *et al.* 1973, Cheah and Osborne 1978). Roberts *et al.* (1973), also using rye seeds, demonstrated that degraded rRNA and a problem with transferase I, which is involved with the binding of the aminoacyl-tRNA to the ribosome, contributed to lack of protein synthesis in non-viable embryos during imbibition (Roberts *et al.* 1973, Roberts and Osborne 1973).

During ripening on the mother plant, cells undergo DNA synthesis to form nuclei with a 4C content which is then halved during cell division. If seeds are harvested too early or the seed moisture content drops below a critical level, then cell division may not occur and the seeds may arrest at the G_2 stage in the cell cycle (Brunori 1967). Wheat embryos which were left for longer on the mother plant showed higher incorporation rates of labelled leucine into protein and thymidine into DNA during early germination (Dell'Aquila and Tritto 1991). Mature carrot seeds possess more protein and nucleic acid per unit of dry matter in comparison with immature seeds (Brocklehurst and Dearman 1980). Carrot seeds which were harvested too early and showed reduced vigour, possessed rRNA which was more susceptible to degradation by artificial ageing than mature seeds (Thompson *et al.* 1987). During imbibition, RNA is degraded and synthesised. In mature carrot seeds, rRNA synthesis outweighs degradation earlier in comparison with immature seeds (Thompson *et al.* 1987).

The effect of long-term storage on seeds and inducing vigour differences in a seedlot is often studied by artificially ageing (eg. Gaidarzhieva *et al.* 1991) the seeds at high temperatures (eg. 40-50°C) and moisture contents. Nucleic acid metabolism during seed deterioration has been reviewed by Cherry and Skadsen (1986). Aged seeds showed a lower incorporation of ^{14}C-leucine, ^{3}H-uridine and ^{3}H-thymidine in comparison with unaged seeds of pea (Barker and Bray 1972), wheat (Dell'Aquilla and Tritto 1991) and maize (Gaidarzhieva *et al.* 1991). The incorporation of [6- ^{3}H]-thymidine into DNA decreased with the increasing time of the ageing treatment in wheat seeds (Dell'Aquila and Margiotta 1986). Artificially ageing seeds also

delayed the onset of DNA replication by 24 hours (Thornton *et al.* 1993) and delayed mitotic activity (Dell'Aquilla and Margiotta 1986). Artificial ageing disrupts metabolism but if the treatment is not too harsh, then viability is not affected and the repair of membrane damage is possible (Berjak and Villiers 1972). As the length of the ageing treatment increases, more damage is done. For example, the ability of the cell-free translation system extracted from aged wheat embryos to translate RNA decreases with the length of the ageing treatment (Noubhani and Gidrol 1992). Artificially ageing seeds may not reflect the true changes which occur with natural ageing. For example, the relative proportions of different phospholipids present in accelerated aged seeds are different to those in naturally aged seeds (Koostra and Harrington 1969) although this may be due to the extreme reduction in viability by the artificial ageing treatment.

1.7.2. **Nucleic acid integrity**

RNA species are not repaired and are less stable than double stranded DNA. RNA has a higher probability of base degradation because the presence of the 2'-hydroxyl group of ribose makes the phosphodiester bonds very susceptible to hydrolysis (rev. Lindahl 1993). DNA has additional properties which aid in stability, for example, the presence of histones. This stability of DNA was demonstrated in embryos which had been dead for at least seven years by the fact that most nucleosome structure still remained (Cheah and Osborne 1977). The double helical structure of DNA also increases the half-life of bases because it has been calculated that individual cytosine residues in single stranded DNA have a half-life of 200 years whilst in double stranded DNA, hydrolytic cytosine deamination occurs at only 0.5-0.7% of the rate of the single stranded DNA in solution (Lindahl 1993).

The integrity of RNA and DNA is important for the development and growth of the seedling. Brocklehurst and Fraser (1980) demonstrated that a loss of rRNA integrity was associated with impaired germination performance and loss of vigour. In addition, loss of desiccation tolerance in primed leek seeds is accompanied by increased rRNA degradation during rehydration with subsequent poor germination (Davison *et al.* 1991). Immature seeds also had less intact rRNA than mature seeds (Brocklehurst and Fraser 1980). Osmopriming, which improved seed performance, facilitated the replacement of damaged rRNA in low vigour leek seeds with an increase in large rRNA species (Davison *et al.* 1991).

The synthesis of normal RNA species is dependent on intact DNA. Sen and Osborne (1977) and Bray and Dasgupta (1976) showed that in aged seeds only low molecular weight RNA is transcribed. This may be due to the fragmentation of the DNA template not allowing messages of greater length to be synthesised (Priestley 1986). The presence of chromosomal and chromatid aberrations in plants grown from ageing seeds has been recognised for many years (eg. Nichols 1942) and have been comprehensively reviewed by Roos (1982) and Priestley (1986). Factors such as X-rays (Nichols 1942), increased temperature, increased seed moisture content (Villiers and Edgcumbe 1975) and an increased oxygen level have been shown to be associated with the accumulation of aberrant cells (Abdalla and Roberts 1968). When nuclear damage reaches a critical level then the seeds are no longer capable of germinating (Abdalla and Roberts 1968). Priming in PEG solution or water and humidification in air decrease the frequency of chromosomal aberrations in lettuce (Villiers and Edgcumbe 1975) and pea (Sivritepe and Dourado 1995). This could be explained by the uptake of water by the seed allowing some kind of repair of the DNA, so that genetic damage is less frequent and chromosomal and chromatid aberrations are less likely to be observed when the cells divide at germination.

1.7.2.1. DNA repair in seeds

DNA repair in seeds has been reviewed by Osborne *et al.* (1984). A low level of DNA synthesis which precedes that regarded as replicative synthesis has been detected within 15 minutes of germination in rye embryos (Osborne *et al.* 1980/81). Using BND cellulose and labelled thymidine, the extent of radioactivity in single stranded and double stranded DNA can be estimated. If rye embryos are irradiated to induce DNA strand breaks, imbibed for 50 minutes and pulse-labelled with [Me-^{3}H]-thymidine in the last 20 or 30 minutes, then most radioactivity is eluted in the fraction which is associated with double stranded DNA (Osborne *et al.* 1980/81, 1984, Osborne 1983, Vázquez-Ramos and Osborne 1986). This is described by Osborne (1988) as unscheduled non-replicative synthesis. It is non-replicating synthesis because an inhibitor of semi-conservative replication, abscisic acid, did not affect the elution peaks during a two hour imbibition of non-irradiated embryos (Vázquez-Ramos and Osborne 1986). This labelled thymidine incorporated into the DNA is stable because even when the pulse was chased for three hours there was no change (Vázquez-Ramos and Osborne 1986).

In addition, the fragmentation profile of DNA from embryos imbibed for two hours is the same as if the seeds had been imbibed then dried back (Osborne 1988).

An autoradiographic method of investigating DNA strand breaks in thin sections is to incorporate ^{3}H-dCMP to any free 3'OH termini using 3'-terminal deoxynucleotidyl transferase to produce silver grains on processing (Cheah and Osborne 1978). Following irradiation, a higher percentage of silver grains per nucleus was found in comparison with untreated cells (Osborne *et al.* 1980/81). During the first few hours of the osmopriming of leek seeds, a low level of DNA synthesis is seen (Ashraf and Bray 1993). This has been described as DNA repair and also DNA replication occurring in the mitochondria (Ashraf and Bray 1993). DNA synthesis occurring in organelles (Vázquez-Ramos and Osborne 1986) may be a possible explanation for a third type of DNA biosynthesis, apart from nuclear semi-conservative replication and repair, which has been described as occurring in early germination. This third type of DNA biosynthesis has also been investigated by Bucholc and Buchowicz (1992) who found a rapid synthesis of telomere-related sequences in the extrachromosomal fraction during early germination.

To study DNA repair systems in seeds, the DNA can be artificially damaged to produce mostly single strand breaks using γ-irradiation. The greater the dose of γ-irradiation, the higher the incorporation of ^{3}H-methyl-thymidine incorporation during imbibition (Osborne *et al.* 1980/81). DNA fragments can be separated by centrifugation in an alkaline sucrose gradient (Osborne *et al.* 1980/81) or in a neutral sucrose gradient (Cheah and Osborne 1978) or electrophoretic fractionation on an alkaline agarose gel (Elder *et al.* 1987, Osborne 1988). In the latter technique there is a movement of the DNA profile of irradiated embryos to a lower mean molecular weight in comparison with DNA extracted from untreated embryos (Elder and Osborne 1993). Following a period of imbibition, fragments are converted back to higher molecular weight by DNA repair. This has been shown in barley (Tano and Yamaguchi 1977) and rye (Elder *et al.* 1987).

It is thought that the repair of single strand DNA breaks occurs by a patch containing the incorrect nucleotide(s) being excised by an exonuclease, which is then replaced by polymerase activity using the alternate strand as a template and rejoined by DNA ligase activity (Osborne 1983, 1988). Evidence for this includes the fact that these enzymes, implicated in natural repair

processes, have been found in harvested seeds. Work by Dandoy *et al.* (1987) suggests that in the process of the repair of base damage generated during seed storage, apurinic or apyrimidinic sites are produced by DNA glycosylases. In addition, Daniel and coworkers (Daniel *et al.* 1985, Daniel and Bryant 1988) have found two forms of DNA ligase in pea seedlings which may show similar characteristics to ligase I and II found in mammals. A review of plant DNA polymerases was carried out by Litvak and Castroviejo (1985). Aphidicolin, which inhibits α-polymerase activity, does not affect ^{3}H-thymidine incorporation greatly until eight hours of imbibition has occurred indicating that DNA replication is not a major event in the first hours of germination (Osborne 1988). In addition, low levels of DNA synthesis were not inhibited by the presence of aphidicolin during the first three days of osmopriming (Ashraf and Bray 1993). β-polymerase activity, which has been implicated in DNA repair, is inhibited using dideoxythymidine triphosphate (ddTTP) by almost 50% in the first three hours of imbibition (Elder and Osborne 1993). DNA topoisomerases break and reseal phosphodiester bonds which may help in maintaining DNA integrity and these have been found in maize embryos and carrot cells in a suspension culture (Carbonera *et al.* 1995). DNA repair of induced damage by irradiation has even been shown to occur in dormant imbibed seeds of rye and oats (Osborne 1985, Elder and Osborne 1993).

1.7.2.2. DNA integrity in relation to seed quality

Decreasing vigour and viability affects DNA integrity in two ways. Firstly, DNA strand breaks (and other damage) occur during the loss of viability in rye (Cheah and Osborne 1978, Osborne *et al.* 1980/81) and maize (Vázquez-Ramos *et al.* 1988). This is shown by the amount of extractable high molecular weight DNA decreasing with viability without a change in total DNA (Osborne *et al.* 1980/81). Also greater DNA fragmentation is apparent when extracted DNA is denatured and run on alkaline agarose gels (Elder *et al.* 1987). Secondly, the DNA repair capacity declines with decreasing viability (Elder *et al.* 1987). DNA ligase function is decreased in aged rye (Elder *et al.* 1987, Osborne 1988) and maize embryos (Vázquez *et al.* 1991). A decrease in DNA polymerase activity is also seen in deteriorated maize (Vázquez-Ramos *et al.* 1988) and barley embryos (Yamaguchi *et al.* 1978). High vigour/viability embryos possess DNA with very little damage and effective repair systems. Low viability and vigour seeds show a delay in the start of DNA replication and before the first cell division (Osborne *et al.* 1980/81). This might indicate that the DNA of low quality

seeds needs repairing first before germination can proceed to radicle emergence.

1.7.3. **Aims**

The aim of this section of work was to determine if DNA repair was detectable in sugar-beet embryos by fractionating the DNA on alkaline agarose gels and if so, how vigour affects the capacity of embryos to repair artificially induced DNA damage under optimal conditions. The extent of DNA repair under sub-optimal conditions for two different vigour levels was also measured because differences between vigour levels were greatest at sub-optimal conditions for wheat seedlots (Blowers *et al.* 1980). With DNA extraction or pulse-labelling experiments, it is not feasible to determine the DNA amounts per nucleus (Bino *et al.* 1992). Therefore nuclear replication steps in seeds have been identified using Feulgen staining or more recently, flow cytometry. In the present work, the effect of the seed treatments, advancement and artificial ageing, and also the vigour level of the embryos on the DNA status has been investigated using Feulgen staining and image analysis.

2.1. SEED MATERIAL

The aim of this work was to investigate seed vigour. It was decided that seedlots which had different vigour levels but similar viabilities would be used and to use growers lots from the same cultivars. It was also important that all seedlots had at least 80% viability because anything less generates an undesirable degree of variability between sub-samples (Thomson 1963). A growers lot is produced by one farmer on one site and a number of growers lots are mixed together to form a commercial seed bulk. A growers lot will be referred to as a seedlot in this thesis. The cultivars used are described in Table 2.1. From one seedlot of cv. Planet, lots were prepared by differing degrees of rubbing (footnote 1) and these four lots were then separated into high and medium density (footnote 1) using a gravity table. To study the effect of fruit size on seed vigour, cv. Zulu seeds were graded by diameter into ten 0.25 mm fractions (footnote 2). In addition, commercial seed bulks (obtained by Germain's (UK) Ltd.), harvested in 1992 and 1993, have also been used in order to investigate storage deterioration (section 2.1.1.4.) and ploidy.

TABLE 2.1 Summary of seed material used.

Cultivar	Seed producer	Types of intra-cultivar differences	Ploidy	Year of harvest
Cyrano	SES	10 seedlots (A-J)	triploid	1992
Rizor	SES	10 seedlots (A-J)	diploid	1992
Marathon	Maribo	3 harvest years (A-C)	triploid	1990-1992
Matador	Maribo	4 countries (A-D)	triploid	1992
Planet	Lion	8 types of rubbing (1-4) and grading (a or b)[1]	triploid	1992
Zulu	Hilleshog	10 seed sizes (1-10)[2]	diploid	1992

[1] 1=no rubbing, 2=light rubbing, 3=normal rubbing, 4=heavy rubbing
a = high density, b= medium density

[2] size 1- 10 = small (<3.5 mm) to large (>5.5 mm)

2.1.1. Seed treatments

2.1.1.1. Rubbing

Seedlots used in germination tests and field trials were rubbed using a seed polishing machine in order to remove some of the excess cortex. This is a seed process used commercially which produces a more uniform seed size and incidentally reduces the amount of *P. betae* infected material.

2.1.1.2. Thiram-steeping

Thiram (tetramethyl thiuram disulphide) is used to control *P. betae* in laboratory tests and in the field. Rubbed seeds were steeped in an aqueous suspension of 0.2% (w/v) thiram for 6 h (approximate length of time used commercially) at 25°C while air was bubbled through the suspension. The seeds were heat dried (c.a. 40°C) for 3 h and then left overnight to dry at room temperature.

2.1.1.3. Primed advancement treatment

The primed advancement treatment has been developed at Broom's Barn by Durrant and others (Durrant *et al.* 1993) to devernalise sugar-beet seeds and increase rate of emergence in the field. Rubbed seeds were given the advancement treatment by Germain's (UK) Ltd. using a method developed by Durrant and Mash (1992).

2.1.1.4. Artificial ageing

The sugar-beet seedlots had an average moisture content (mc) of 10%. In order to induce ageing, water was added to achieve a final moisture content of 24% (% of fresh weight) as recommended by ISTA (1981). This was done by placing the seeds in tubes which were sealed with two layers of Parafilm and rolled overnight to equilibriate the moisture. The tubes were placed in a water bath at 45°C (ISTA 1981) for 48 h. ISTA recommends incubating the seeds at 45°C for 24 h but standard germination tests carried out on seeds (cv. Cyrano E), which were deteriorated for a range of times, demonstrated that a 48 h incubation at the same temperature reduced the rate of germination more effectively without decreasing viability to

a large extent (Figure 2.1).

2.2 SEED VIGOUR ASSESSMENT

Table 2.2 summarises the methods of seed vigour assessment used on the different types of seeds.

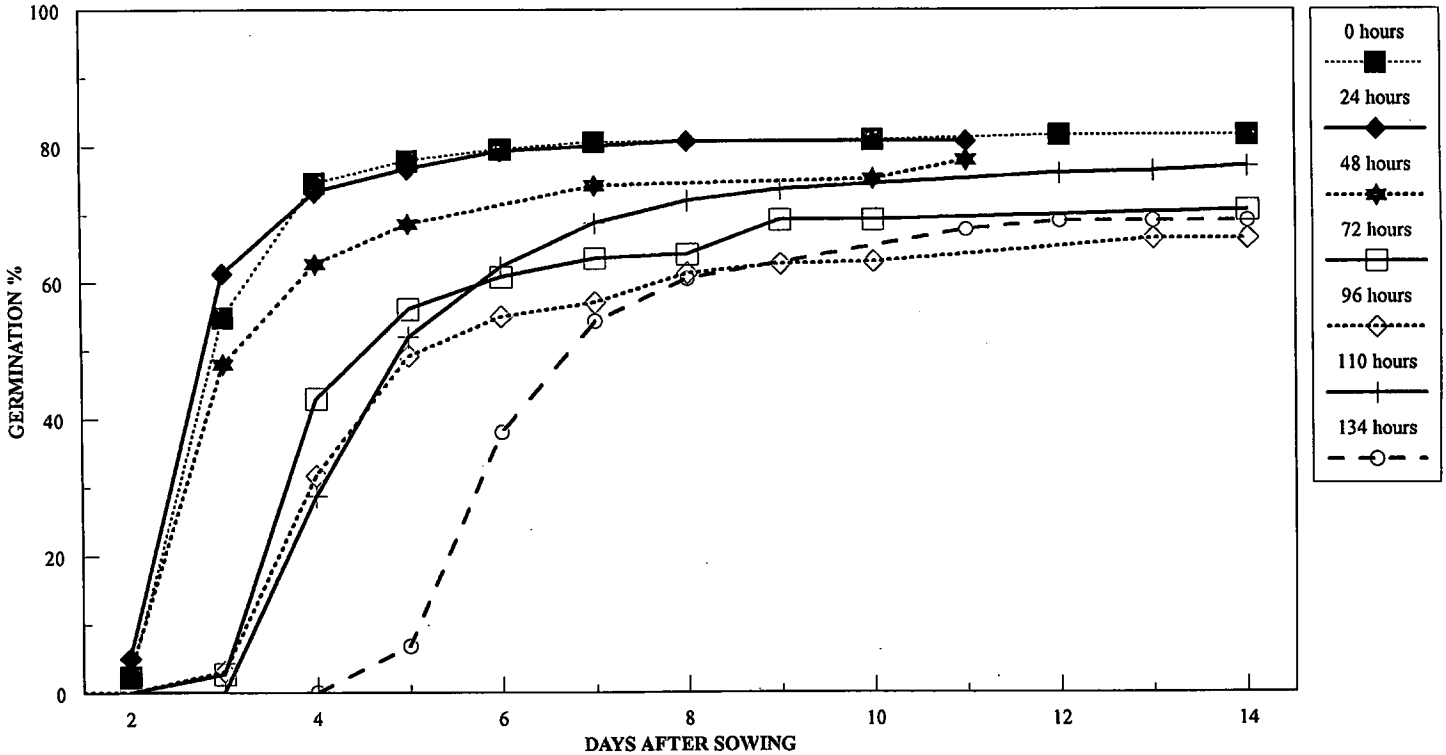
- For the seedlots 1 experiment, four cultivars, each with a number of lots, were selected. The aim was to assess the vigour of these lots using a range of germination tests (section 2.2.2.) and the RNA/DNA ratio measurement (section 2.2.3.) and compare these results with vigour assessed in the field (section 2.2.4.). The cv. Rizor lots were assessed separately from the other lots in this experiment.

- To enable a more thorough investigation of seed vigour (seedlots 2 experiment), seedlots exhibiting the most extreme differences in vigour levels in three of the four cultivars were selected using the data from the seedlots 1 experiment. The seeds were the same as those used in the seedlots 1 experiment except that the seedlots, B and D of cv. Matador were newly supplied by the seed company. The seedlots were assessed by germination tests and more replicates were used to determine the RNA/DNA ratio. In addition, an improved method for determining the RNA/DNA ratio was used (method 2). Field trial 4 (section 2.2.4.2.) had more replicates than trials 1 to 3 because there were fewer lots to assess. In this trial, seedlings were harvested at establishment to determine whether seed vigour effects were reflected in the subsequent seedling development.

- Improvements in seed vigour by the advancement treatment have been demonstrated through accelerated seedling emergence in the field (Thomas *et al.* 1993). An experiment (steeped-advanced) was carried out to determine whether this vigour improvement, in comparison with thiram-steeped seeds, was detectable using germination tests, the RNA/DNA ratio and field trials (trial 2).

- The effect of rubbing and grading on field performance (trial 3) was assessed using cv. Planet seeds. No other tests were carried out on these seeds.

FIGURE 2.1 The effect of the length of the artificial ageing period on the germination of cv. Cyrano E at 20°C



- Experiments were set up to determine the effect of certain variables on the RNA/DNA ratio (Table 2.3). These were: fruit size, the ploidy level of the cultivar and treatments such as advancing, thiram-steeping and artificial ageing. To investigate the effect of ploidy, three pairs of bulks from commercial cultivars were selected so that each pair contained a diploid and a triploid bulk which had similar viabilities (on the basis of 7 and 14 day germination test data provided by Germain's (UK) Ltd.). In addition a further pair of seed bulks was selected which had different viabilities but the same ploidy (triploid).

2.2.1. **Calculations using germination and emergence data**

The mean time to germination (MGT) was calculated as follows:

$$\frac{\Sigma (d \times n)}{\Sigma n}$$

where d = number of days from sowing, n = number of seeds germinated on day d (Ellis and Roberts 1981). For some germination tests, the number of hours instead of days was used. An additional measurement included counting the number of seedlings with hypocotyls longer than 2 cm (Durrant and Loads 1987). The mean time for hypocotyl elongation ie. the hypocotyl being longer than 2 cm (MHT) was calculated using the same formula as MGT but n equals the number of seedlings with hypocotyls larger than 2 cm on day d. The mean time to emergence (MET) in the field can be calculated using the same formula where n is the number of seedlings that have emerged in the field on day d.

Time to 50% germination or emergence (T_{50}) is expressed as the time in days or hours when 50% of the seeds planted have germinated. In addition, T_{50}* is expressed as the time in days or hours when 50% of the final count of germinating seeds have germinated. Time to 30% germination (T_{30}) was also determined. The T_{50}, T_{50}* and T_{30} are calculated by the interpolation of germination curves generated by the germination test or field trial.

TABLE 2.2 Summary of the method of seed vigour assessment and seed treatments for each experiment.

Experiment	Lots used	Germination tests (GT)	RNA/DNA	Field trials	Seed treatments
seedlots 1	Cyrano A-J Marathon A-C Matador A-D Rizor A-J	all	method 1	trial 1 trial 1 trial 1 trial 3	rubbed and thiram-steeped for trials and GTs, raw seeds for RNA/DNA
seedlots 2	Cyrano G,H Matador B,D Rizor F,B	all except 15°C GT	method 2	trial 4	all seeds rubbed and sieved (3 mm-4.25 mm), thiram-steeped seeds for field trials and GTs
steeped/ advanced	Cyrano B,G,H	cold stress and 20°C GT only	method 1	trial 2	all seeds rubbed and thiram-steeped or advanced
rubbed/ graded	Planet 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b	none	none	trial 3	all seeds thiram-steeped

TABLE 2.3 Summary of the seed material and treatments used to study factors which may affect the RNA/DNA ratio.

Factor	Treatments and material	Cultivars and lots	Number of replicates
seed treatments 1	untreated thiram-steeped advanced	Cyrano B, G, H	4
seed treatments 2	untreated advanced aged advanced-aged	Cyrano H	6
fruit size	fractions 1,3,5,7,9,10	Zulu	6 (2 x 3 replicate experiments)
genotype	triploid (t) diploid (d)	Regina 322(t) Roberta 342(d) Zulu 333(t) Saxon 316(d) Rose 371(t) Druid 336(d) Rex 321(t) Hilma 320(t)	3

2.2.2. Laboratory germination tests

The germination tests which were used to assess the cultivars are summarised in Table 2.2 and are described in more detail below.

2.2.2.1. Standard germination test

As described by ISTA (1990), the standard germination test used a pleated filter paper and a paper cover (Anderman and Co., 145 London Rd., Kingston, Surrey, KT2 6NH) in an air-tight plastic box. The paper was dampened with 35 ml of distilled water to produce 48% paper saturation (Table 2.4) and left to equilibriate overnight. Two intact seeds were placed in each pleat so that there was a total of 100 seeds per box. Three replicates were used and all of the boxes were placed randomly in an incubator at 20°C. A seed was recorded as germinated once the root tip was clearly visible, ie. at least 2 mm long (Durrant and Jaggard 1988). Germinated seeds were counted on day 3, day 6 and day 14. The percentages of infected and abnormal seedlings were calculated following recommendations by ISTA (1979). Abnormal seedlings were not counted as having germinated.

A standard germination test was also carried out at 15°C since this temperature is frequently used by seed testers. Although ISTA recommends first counting on day 4, the number of seeds which had germinated was counted daily for 17 days. The number of seedlings with the hypocotyl larger than 2 cm was also counted daily (Durrant and Loads 1987). Using the data collected, the T_{50}, MGT and MHT were calculated.

2.2.2.2. Cold stress test

The cold stress test was identical to the standard germination test at 15°C except that the seeds were incubated at 9°C and counting was continued for an additional 14 days because germination was much slower. T_{50} , T_{50}* (seedlots 2 experiment only), MGT and MHT were calculated.

2.2.2.3. Wet stress test

The wet stress test was the same as the standard germination test at 20°C but 60 ml of water (Lovato and Cagalli 1992) was added to each box instead of 35 ml. Following the equation in Table 2.4, the substrate saturation was calculated as 83% which was 35% more than the standard test. The percentage of seeds which had germinated and the number of hypocotyls larger than 2 cm were counted on day 2 (for seedlots 2 experiment only), day 3 (cv. Rizor seedlots only), day 4, day 7 and day 14.

TABLE 2.4 The determination of water quantity per box for the standard germination test (following the method of Jassem *et al.* 1993). Using the following formula it is possible to calculate the substrate saturation %:

$$N=\frac{a \times b}{100} \qquad 35=\frac{72.47 \times a}{100} \quad \text{therefore } a=48.3\%$$

where: N-amount of water (ml)/box
a-substrate saturation %
b-maximum water capacity (ml) [1]

Replicate	Mass (g) of box, filter paper (a)	Mass (g) of saturated box and paper	Maximum water capacity (g)
1	100.16	169.98	69.82
2	100.58	173.81	73.23
3	99.42	170.61	71.19
4	100.02	175.66	75.64
		mean	72.47

[1] determined by filling a pre-weighed box and paper with water, pouring off the excess water after 10 minutes and re-weighing

2.2.2.4. Cold sand test

The cold sand test was adapted from the method by Lovato and Cagalli (1992). The sand was moistened to 60% of the maximum water holding capacity (w.h.c.) rather than the 40% w.h.c. used by Lovato and Cagalli. A preliminary experiment had indicated that 60% imposed a

greater germination stress on the seeds improving vigour comparisons. The sand (Foremost Horticultural Silver Sand no. 500053, Croxden Horticultural Products Ltd, Cheadle, Stoke-on-Trent, Staffs.) was sterilised overnight at 85°C. The sand trays were set up by adding 1.5 kg of sand to plastic half-trays (175 cm x 22 cm, Plantpak, Maldon, UK) and then adding 220 ml of water. These trays were covered in a plastic bag and left overnight to equilibriate. Three boxes were prepared for each seedlot. Using a grid with evenly spaced holes, 100 whole seeds were placed in each box. The sand surface was then flattened with a piece of wood. Approximately 350 g of 60% w.h.c. sand, which had been prepared the day before, was added on top of the seeds and then flattened. Each tray was placed back into its original plastic bag and sealed. The seed trays were randomly arranged in a controlled environment room (8°C ± 0.8, no lighting). One week after planting, the temperature was increased to 20°C (19.5°C-21.25°C) and the tungsten and fluorescent lights were switched on (light intensity of c.a. 220 $\mu mol/m^2/s$). The number of emerged seedlings (emergence was defined by the hypocotyl hook emerging through the sand surface) was counted on day 14.

2.2.3. **Nucleic acid synthesis and integrity**

Three experimental approaches were used:

- The ratio of extractable RNA to extractable DNA was calculated for the seedlots and for seeds which had been treated in different ways.
- Feulgen staining was carried out on embryos from seeds which had been treated (advanced and aged) or which had different vigour levels. Feulgen staining allows the number of copies of genetic material to be determined in each cell which indicates their stage in the cell cycle.
- DNA repair in sugar-beet embryos was investigated; particularly the effect of seed vigour on the potential to repair DNA damage during imbibition following γ-irradiation of the dry embryo.

2.2.3.1. Extraction of true seeds and embryos

The commercial sugar-beet 'seed' is botanically termed the 'fruit' and the term 'true seed' refers to the embryo plus its perisperm and integuments (Richard *et al.* 1989). True seeds were

isolated from the seed coat by first prising off the operculum (commonly known as the cap) with a mounted needle and then carefully removing the true seed. The seed coat was removed because the seed cortex contains a range of chemicals, including phenolic compounds (Battle and Whittington 1969), which affects the quantification of nucleic acid in solution (section 2.2.3.3.). Fifty true seeds per microcentrifuge tube were needed for each nucleic acid extraction (section 2.2.3.3.). True seeds which were abnormal and found in clusters or in polyembryonic fruits were not included. For the true seed extraction of seedlots 2 experiment, abnormal true seeds and all true seeds found in polyembryonic fruits were included. Each tube of 50 true seeds was weighed and then frozen in liquid nitrogen. The tubes were stored in a -70°C freezer.

In order to Feulgen stain the nuclei of the embryo root tips, the embryos had to be extracted from the true seeds. Following extraction from the seed coat, the true seed was imbibed in deionised water for 1 h at room temperature in order to soften the surrounding tissue. Using tweezers and a mounted needle, the embryo was removed from the perisperm and integuments. The embryo was then rinsed in deionised water and placed in fixing solution (1:1 acetic acid:ethanol) overnight.

The extracted embryos were also used for the DNA repair (following irradiation) experiments. After rinsing in deionised water, the embryos were placed on filter paper and dried at 24°C overnight. The embryos were stored in microcentrifuge tubes in the refrigerator.

2.2.3.2. Nucleic acid extraction for the RNA/DNA ratio

The RNA/DNA ratio was determined by measuring the amount of extractable high molecular weight (H.M.W.) nucleic acids in true seeds. Method 1 (section 2.2.3.2.1.) was used to calculate the RNA/DNA ratio for seedlots 1 experiment and the steeped-advanced experiment and to study the effect of different variables, such as fruit size, on the RNA/DNA ratio. Method 2 (section 2.2.3.2.2.) was used for the seedlots 2 experiment. Also, for method 1, untreated seeds were used whereas in method 2, the seeds were rubbed and then sieved to remove the extreme sizes.

It is appropriate to mention here that the smaller molecular weight (S.M.W.) DNA and RNA

are lost in all the methods of nucleic acid extraction used. This is shown in Table 2.5 where H.M.W. nucleic acids were extracted from cv. Rizor B true seeds using method 2 (section 2.2.3.2.2.) and the DNA measured using the fluorimeter (method 2) and Hoechst dye (section 2.2.3.3.2.). The ethanol decanted from the nucleic acid pellet (following the overnight precipitation and centrifugation) was incubated with 0.05 vol. trichloroacetic acid (Sigma) overnight to precipitate the S.M.W. DNA. Following a 60 min centrifugation at 13,000 rpm, the pellet was air-dried and then dissolved in 50 µl 0.1 x TE buffer. The concentration of DNA in these samples was measured in the same way as for the H.M.W. DNA. The table shows that the smaller molecular weight DNA, which is not normally extracted, constitutes approximately 10% of total DNA.

TABLE 2.5 The concentration of H.M.W. DNA extracted by standard methods and the amount of S.M.W. DNA left in the ethanol supernatant. The DNA concentration of S.M.W. was halved to give comparable results with the H.M.W. DNA concentration because the S.M.W. DNA was dissolved in 50 µl of 0.1 x TE buffer whereas the latter was dissolved in 100 µl of 0.1 x TE buffer.

Sample	H.M.W. DNA concentration (µg/ml)	S.M.W. DNA concentration (µg/ml)	S.M.W. DNA % of total DNA
A	96.51	11.38	10.54
B	95.97	8.52	8.15
C	87.40	12.12	12.17
		mean	10.29

2.2.3.2.1. *Method 1*

The following chemicals were obtained from Sigma Chemical Company Ltd., Poole, Dorset: phenol:chloroform:isoamyl alcohol (25:24:1); Trizma Base; p-aminosalicylic acid, sodium salt; 2-mercaptoethanol and isoamyl alcohol. Chloroform and sodium acetate were purchased from BDH Laboratory Supplies, Poole, Dorset. Triisopropylnaphthalene sulphonic acid (TNS, sodium salt) was obtained from Kodak, Phase Separations, Deeside Industrial Park, Clwyd and absolute alcohol (100% ethanol) was purchased from Hayman Ltd, Eastways Industrial Park, Witham, Essex. Ethylenediaminetetraacetic acid (EDTA) was obtained from Boehringer

Mannheim UK, Lewes, East Sussex.

The nucleic acid extraction procedure was adapted from the method of Clarke and James (1991). Centrifugations (13,000 r.p.m.) were carried out using a bench microcentrifuge (Micro Centaur, MSE) at room temperature. Inorganic solutions, pipette tips and tubes were autoclaved and latex gloves were used at all times.

Each microcentrifuge tube was taken out of the freezer and kept in liquid nitrogen together with the stainless steel rods used for crushing the samples. Each frozen sample was ground for 2 min and then 350 µl extraction buffer (EB) was added followed by 350 µl of phenol:chloroform:isoamyl alcohol reagent (PR). The extraction buffer contained 50 mol m^{-3} Tris-HCl (pH 8.0), 4% (w/v) p-aminosalicylate, 1% (w/v) TNS and 2% (v/v) β-mercaptoethanol. The tubes were centrifuged for 45-60 min and the supernatant removed and stored on ice. Another 350 µl of EB was added to the homogenate in the original tubes, mixed and centrifuged for 30 min. Again the supernatant was removed and added to the stored aqueous phase. 700 µl of PR was added to each tube of stored aqueous phase and then centrifuged for 15 min. The supernatant was removed and placed in a new tube. 700 µl of chloroform:isoamyl alcohol (24:1) was added to the supernatant and centrifuged for 30 min. The supernatant was removed and to this, 0.1 vol. of 3M sodium acetate (pH 5.5) and 2 vols. of absolute ethanol were added. The tubes were left in the refrigerator overnight to facilitate nucleic acid precipitation.

The tubes were centrifuged for 30 min, the ethanol poured off and the pellet was washed twice in 70% (v/v) ethanol with a 15 min centrifugation. After a final 15 min centrifugation, the ethanol was decanted and the pellet left to air dry. The pellet was redissolved in 100 µl of 0.1 x TE buffer (1 mM Tris HCl , pH 8.0 and 0.1 mM EDTA, pH 8.0 in double deionised water). The nucleic acid solutions were stored at -70°C.

2.2.3.2.2. *Method 2*

This was the same as method 1 except that all centrifugations carried out on day 1 used the Sigma 4K10 centrifuge (16249 g) at 2°C instead of the MSE bench microcentrifuge which was not temperature controlled. Also the 1 mM Tris HCl solution (in the 0.1 x TE buffer) had a

pH of 7.6 rather than 8.0 and the tubes were left at -20°C overnight rather than at 4°C during the ethanol precipitation stage.

2.2.3.3. Nucleic acid quantification for the RNA/DNA ratio

An accurate estimate of the concentration of DNA and RNA in a sample is necessary in order to calculate the RNA/DNA ratio. Certain fluorescent dyes, such as Hoechst 33258, bind specifically to DNA so that the concentration of DNA in a solution can be determined (Weisblum and Haenssler 1974). There are no dyes which bind specifically to RNA as they also bind to DNA (eg. ethidium bromide). DNA and RNA in a solution have been quantified using ethidium bromide by measuring the increase in fluorescence. To quantify the RNA, the nucleic acid solution is incubated with RNase and the difference in fluorescence between the solutions with and without the RNase incubation equals the amount of RNA in the solution (Le Pecq 1971, Le Pecq and Paoletti 1966, Sakano and Kamatani 1992). When this method of quantification was used to determine the RNA/DNA ratio, it was found that the relative increase in fluorescence due to RNA binding with ethidium bromide was much smaller than that for the DNA binding with the ethidium bromide. This meant that the fluorescence due to the ethidium bromide binding to the DNA masked the fluorescence due the ethidium bromide binding to the RNA. Therefore other methods of nucleic acid quantification were developed.

The concentrations of nucleic acids in a solution can be also directly quantified on a spectrophotometer without using a dye/stain because they show a characteristic UV absorption band with a maximum at around 260 nm and a minimum at 230 nm (Müller *et al.* 1993). Nucleic acids obey Beer's Law so that absorbance increases linearly with concentration up to 3.0 optical units (A). The absorbance at 260 nm of different concentrations of calf thymus DNA (Sigma type I, sodium salt) is shown in Figure 2.2 and calf liver RNA (Sigma type IV) in Figure 2.3. Measuring the absorbance at 260 nm of DNA and RNA in the same sample is shown in Figure 2.4.

The absorbance at 260 nm of 1.0 A measured in a cuvette with a 1 cm path length is indicative of double-stranded DNA at a concentration of approximately 50 µg/ml (Berger 1987). Therefore nucleic acid concentrations are determined as follows:

$$1.0\ A_{260} = 50\ \mu g/ml\ ds\ DNA \quad (1)$$

$$1.0\ A_{260} = 33\ \mu g/ml\ ss\ DNA$$

$$1.0\ A_{260} = 40\ \mu g/ml\ RNA \quad (2)$$

Other useful wavelengths to measure for the levels of contamination of nucleic acid solutions are: 230 nm for phenol and proteins; 280 nm for aromates and 320 nm for turbidity. The effect on the UV absorption spectra of including the seed coat during nucleic acid extraction and quantification is shown in Figure 2.5. The level of contaminants generated by the seed coat is reflected by the higher absorbance at 230 nm and at wavelengths between 300 nm and 500 nm. When the seed coats are removed, the extracted nucleic acid solution is much cleaner. Figure 2.6 demonstrates the increase in absorbance by nucleic acids when more true seeds are used per sample. Fifty true seeds per sample were used to ensure that the spectrophotometry readings were more accurate ie. greater than 1.0 A.

Hoechst dye (Sigma, H33258) binds to the DNA bases, particularly A-T rich regions, and this binding is not affected by commonly used laboratory salts and detergent solutions (Cesarone *et al.* 1979). The change in fluorescence when the dye binds to DNA can be detected using a fluorimeter. Using a wavelength scan, the optimum excitation wavelength and emission wavelength can be obtained. The wavelengths used are 360 nm for the excitation and 460 nm for the emission (Vollenweider and Groscurth 1992). Figure 2.7 shows the emission wavelength scan of Hoechst dye and Hoechst dye bound to DNA using the excitation wavelength at 360 nm.

2.2.3.3.1. *Method 1*

Total nucleic acid concentration was measured using a UV/Vis spectrophotometer (Unicam 8700 series). Each sample was vortexed before pipetting 20 µl of the sample into 1 ml of 1 x TNE (0.1 M NaCl, 10 mM Tris-Cl, 1 mM EDTA pH 7.4) in a semi-micro self-masking UV silica cell (path length 10 mm). The mean was calculated from three separate replicates of each sample. The baseline was produced using 1 x TNE buffer only. The absorbance was measured at 230 nm, 260 nm, 280 nm and 320 nm using a deuterium light source.

The DNA concentration was measured using a fluorimeter (Hitachi fluorescence

spectrophotometer model F-2000, bandpass 10/5 nm). A calibration curve was generated using four DNA standards made of calf thymus DNA. For each standard and sample, 2 µl of vortexed DNA standard or sample and 1 ml of working dye solution (1 µg/ml Hoechst 33258 in 1 x TNE) were mixed together in a quartz fluorescence cell. The excitation wavelength was set at 360 nm and the emission wavelength at 460 nm generated by a xenon lamp. Measurements were taken after 1 h at 37°C in the dark because the readings of the replicates were more consistent. The exceptions were for the earlier experiments (seedlots 1 experiment and the first three replicates of the size experiment) where measurements were taken immediately. The mean was calculated from three separate replicates of each sample.

The **RNA/DNA ratio** was calculated as follows:

Total nucleic acid (A_{260}) measured on spectrophotometer = a

DNA concentration (µg/ml) measured on fluorimeter=b

Following (1), the equivalent absorbance of the DNA on the spectrophotometer (c) is

$$c\ (A_{260}) = b / 50 \qquad (3)$$

Therefore, the absorbance of RNA (d) can be calculated as follows:

$$d\ (A_{260}) = a - c \qquad (4)$$

To calculate the RNA concentration in µg/ml (e), the dilution factor (20 µl sample in a total volume of 1020 µl) and absorption coefficient (see equation (2)) are taken into account:

$$e\ (\mu g/ml) = d \times 51 \times 40 \qquad (5)$$

Therefore the RNA/DNA ratio is calculated using e and b:

$$RNA/DNA = e / b \qquad (6)$$

This is the first method of calculating the RNA/DNA ratio and is termed RNA/DNA 1.

Alternatively, nucleic acid concentrations (f) in µg/ml can be calculated from absorption readings at 260 nm and 280 nm using the Warburg-Christian formula (Müller *et al.* 1993) to account for contaminating proteins:

$$f = 62.9 \times A_{260} - 36 \times A_{280} \quad (7)$$

The RNA concentration (g) can then be determined by subtracting the DNA concentration measured on the fluorimeter (b) from the total nucleic acid concentration (f):

$$g = (f \times 51) - b \quad (8)$$

The RNA/DNA ratio (RNA/DNA 2) is then calculated as follows:

$$RNA/DNA = g / b \quad (9)$$

Three additional RNA/DNA ratios can be calculated using spectrophotometer absorbance readings at 230 nm (RNA/DNA 3) and 320 nm (RNA/DNA 4). For each calculation, the absorbance at the additional wavelength is subtracted from the total nucleic acid reading at 260 nm (a) and then calculated using equations 5 and 6. Absorbance at 230 nm is used to estimate levels of contamination due to phenolics and proteins and at 320 nm for turbidity because nucleic acids do not absorb light of this wavelength.

2.2.3.3.2. *Method 2*

Total nucleic acid concentration was measured in the same way as for method 1 except that 5 µl of sample and 95 µl of 1 x TNE were placed in an ultra-micro UV silica cell and the samples were mixed using a pipette tip not by vortexing. The baseline used was 5 µl of 0.1 x TE (see nucleic acid extraction method) plus 95 µl of 1 x TNE.

The DNA concentration measurement on the fluorimeter used 995 µl of working dye solution with 5 µl of the DNA standard or sample which was then left at 37°C in the dark for 1 h before measuring. The standards (calf thymus DNA dissolved in 0.1 x TE) used to generate the calibration curve were 0 µg/ml, 50 µg/ml, 100 µg/ml, 250 µg/ml and 500 µg/ml of DNA

(Figure 2.8). The mean was calculated from three separate replicates of each standard or sample used and the samples were mixed using a pipette tip. The DNA standards were also measured on the UV/Vis spectrophotometer in order to generate a graph from which c (A_{260}) can be determined (Figure 2.9).

The **RNA/DNA** ratios can be calculated in a number of ways:

a) RNA/DNA 1-as for method 1 except that the dilution factor in equation (5) is 20 rather than 51;

b) RNA/DNA 2-using the Warburg-Christian equation (7) with equations (8) and (9);

c) RNA/DNA 3 and 4-calculated by subtracting 230 nm or 320 nm from the total nucleic acid absorbance (a);

d) RNA/DNA 5-using a graph (Figure 2.9) of the DNA standards measured on the UV/Vis spectrophotometer and the fluorimeter to calculate the equivalent absorption at 260 nm (c) from the readings determined on the fluorimeter (b). Therefore the absorption of RNA (d) can be calculated as:

$$d\ (A_{260}) = a - c \qquad (10)$$

To calculate the ratio of RNA /DNA 5 in absorption units:

$$RNA/DNA = d / c \qquad (11)$$

e) RNA/DNA 6-the absorption units of RNA and DNA calculated for RNA/DNA 5 can be multiplied by their respective absorption coefficients to give approximate concentrations:

following (2): $RNA\ (\mu g/ml) = d \times 40$ (12)

following (1): $DNA\ (\mu g/ml) = c \times 50$ (13)

Therefore the RNA/DNA ratio 6 can be calculated as:

$$RNA/DNA = \frac{d \times 40}{c \times 50} \qquad (14)$$

f) RNA/DNA 7 and 8-same calculations as for RNA/DNA 5 and 6, using the graph of DNA

standards (Figure 2.9), except that the absorbance reading at 230 nm on the spectrophotometer is subtracted from the total nucleic absorbance at 260 nm;

g) RNA/DNA 9 and 10-same calculations as for RNA/DNA 5 and 6, using the graph of DNA standards (Figure 2.9), except that the absorbance reading at 320 nm on the spectrophotometer is subtracted from the total nucleic absorbance at 260 nm.

2.2.3.4. DNA synthesis

The aim was to assess at which stage in the cell-cycle the embryo root tip nuclei were held following seed treatments. Embryos were extracted from cv. Cyrano G seeds which had been variously pre-treated. The root tip nuclei from untreated seeds, aged seeds, advanced seeds and advanced then aged seeds were then Feulgen stained.

Two other untreated cv. Cyrano seedlots, in addition to cv. Cyrano G, were selected for Feulgen staining based on their different vigour levels when assessed by the RNA/DNA ratio, germination tests and the field trial.

In order to compare leaf tissue nuclei with embryo root tip nuclei, tissue was collected from the cotyledons of a 4 d-old seedling germinated from cv. Cyrano G seeds at 30°C. Nuclei from 5 d-old onion (*Allium cepa*) root tips were used as the standard for estimating the amount of DNA per cell following Feulgen staining.

2.2.3.4.1. *Method*

The method of Feulgen staining was adapted from De Tomasi (1936). The staining technique was optimised for sugar-beet embryos by varying the length of time for fixing, acid-hydrolysis and staining of embryo nuclei in Feulgen solution and then examining the nuclei under oil immersion using a microscope.

The following chemicals were obtained from BDH Laboratory Supplies, Poole, Dorset: potassium metabisulphite, Fuchsin basic standard stain and activated charcoal. Trichloroacetic acid and acetic acid were purchased from Prolabo, Rhone-Poulenc Ltd., Manchester and ethanol was bought from Hayman Ltd., Witham, Essex. The mounting fluid, Euparal, was

obtained from Raymond A. Lamb, Wembley, Middlesex.

The Feulgen stain was prepared by the method of Deitch (1966). Boiled distilled water (100 ml) was added to 0.5 g of Fuchsin basic stain, shaken thoroughly and cooled to 50°C. The stain solution was filtered through filter paper (Whatman 1 qualitative) into a clean amber bottle. 10 ml of 1 N HCl solution and 2 g of potassium metabisulphite were added to the filtrate, the bottle stoppered and then shaken. The stain solution was stored for 24 h in the dark at room temperature and then 0.25 g of activated charcoal, prepared by heating at 100°C overnight, was added. The solution was shaken for 1 min before filtering through filter paper into an amber bottle. The solution was stored in the refrigerator.

The embryos and leaf tissue were stained and mounted on slides as follows. All tissue for staining was fixed overnight in acetic acid-ethanol (1:1) solution and then rinsed in distilled water. The tissues were hydrolysed in 5 N HCl for 30 min at 24°C and rinsed again in distilled water. The embryos and leaf tissue were stained for 1 h in Feulgen solution at 24°C in the dark. Unbound stain was rinsed out of the tissue using three washes of freshly prepared SO_2 water (0.5% (w/v) potassium metabisulphite in 0.05 N HCl solution) followed by rinsing in distilled water. The embryos and leaf tissue were placed in 5% (w/v) trichloroacetic acid for 30 min at 4°C and then rinsed in distilled water. The root tips were sliced from the embryo using a razor blade. The leaf tissue and root tips were placed on ethanol-washed glass slides in drops of distilled water. The tissues were squashed using the end of a mounted needle and left to dry. To mount the squashes, a drop of Euparal was added and a glass coverslip lowered carefully to reduce air bubbles caught under the coverslip. The slides of Feulgen stained nuclei were stored in the dark.

Four slides were prepared for each tissue. Another four slides for each treatment were prepared by repeating the above procedure on fresh tissue except that the leaf tissue was taken from a 7 day-old seedling and the onion from root tips which had been growing for 8 days.

2.2.3.4.2. *Quantification*

The intensity of staining of the nuclei was quantified using image analysis. Each slide was magnified (x 400) under a microscope (Microstar IV-Diastar, Cambridge Instruments, NY)

and the image transferred to the computer using a video camera (CCD colour camera system 1K-M48PK, Toshiba) focused on the microscope eye-piece. The image analysis program (Aequitas IDA, Dynamic Data Links Ltd, Cambridge) was set-up with specific brightness and contrast levels. A scale on a glass slide was used to calibrate the microscope and image analyser. The average intensity and detected area of the stained nucleus and the background intensity were measured for 100 nuclei (25 nuclei per slide) for each treatment and tissue. Three slides for each treatment from the first Feulgen staining and one slide from the second batch of staining were measured.

The intensity readings were determined by calculating the difference between the background readings and the average intensity readings of each nucleus. The DNA content per nucleus (in arbitary units) was calculated by multiplying the intensity of the stained nucleus by the detected area. It was then possible to calculate the percentage of nuclei with each DNA content for each treatment and tissue. The nuclei of *A. cepa* normally have a 2C DNA content of 33.5 pg per nucleus (Bennett and Smith 1976). Therefore the amounts of DNA per nucleus of 2C cells for the stained sugar-beet nuclei were calculated using the onion standard. The percentage of nuclei with a 4C DNA content was also determined for each treatment and tissue.

2.2.3.5. DNA repair

The ability of embryos to repair damaged DNA after γ-irradiation can be investigated using alkaline gel electrophoresis (Elder *et al.* 1987). Imbibition should allow time for the embryo to repair its damaged DNA. The first experiment, using cv. Cyrano C embryos, investigated the feasibility of using these techniques to study DNA repair in sugar beet. The second experiment was set up to compare the degree of DNA repair, following γ-irradiation, at an optimal temperature in two cv. Rizor seedlots of different vigour levels. Two cv. Cyrano seedlots were also selected based on their differing performance in a germination test at 9°C but similar viability at 20°C. The aim of the experiment was to compare their DNA repair capability, following γ-irradiation, at a sub-optimal temperature of 9°C.

2.2.3.5.1. *Preparation of material*

The embryos were either untreated (control) or irradiated; half of the irradiated embryos were also imbibed and dried. Dry embryos were exposed to γ-irradiation of 100 Kr for 105 min (1000 Grays) from a ^{137}Cs-source. The embryos were imbibed on 5.5 cm Whatman 1 filter paper discs soaked in 1 ml of 1.5 % sucrose solution for 2 h at 24°C. The embyos were dried back to their original dry weight at 24°C. All the dry embryos were stored in the refrigerator. Ten embryos per microcentrifuge tube were used for the extraction of DNA.

In order to assess the viability of the embryos, four embryos from each treatment were placed on a small filter paper disc soaked in 1 ml of 1.5% sucrose solution in a small petri dish (5 cm in diameter from Sterilin, Teddington, Middlesex). The lid was sealed and the embryos were left to germinate at 24°C. Four days after beginning the germination test, the health of each embryo was quantified as follows:

0 embryo
1 slight differentiation, no yellowing of leaves
2 differentiation, no yellowing of leaves
3 differentiation, yellowing of leaves
4 well developed radicle and leaves, yellowing of leaves

The treatments for the embryos from the two cv. Cyrano seedlots were the same as above except that imbibition took place at 9°C for 2 h. Also, the embryos were dried back in a desiccator at 9°C and then once the embryos were close to their original weight, dried at 24°C.

2.2.3.5.2. *Method*

All chemicals and enzymes used were purchased from Sigma Chemical Company Ltd., Fancy Rd., Poole, Dorset except for acetic acid, HCl and sodium dodecyl sulphate which were bought from BDH Laboratory Supplies, Poole, Dorset and absolute alcohol from Hayman Ltd., Witham, Essex. Double deionised water was used throughout. Inorganic solutions, pipette tips and tubes were autoclaved and latex gloves were used at all times. Centrifugations were carried out using a bench microcentrifuge (Micro Centaur, MSE) at room temperature.

The method of DNA extraction and alkaline gel electrophoresis was adapted from Elder *et al.* (1987). The embryos in each 0.5 ml microcentrifuge tube were frozen in liquid nitrogen before grinding in 50 µl of homogenising medium (0.15 M NaCl, 0.1 M EDTA, 0.5 % (w/v) SDS in 50 mM Tris-HCl (pH 7.8) solution) using a metal crusher. An additional 400 µl of homogenising medium was added and the grinding continued. The samples were spun at 6,500 r.p.m for 1 min and the supernatant pipetted into clean 1.5 ml microcentrifuge tubes. 50 µl of sodium hydroxide (1 M) and 5 µl of 20 mg/ml proteinase K (from *Tritirachium album*) were added to each sample before incubating at 37°C for 3 h. 30 µl of 3 M sodium acetate in 1 mM EDTA (pH 5.2) solution were added to the solution followed by 1 ml of ice-cold absolute alcohol. The tubes were left overnight at -20°C.

The microcentrifuge tubes were centrifuged at 13,000 r.p.m. for 5 min, the ethanol poured off and the pellet left to air dry. The dry pellet was dissolved in 20 µl of 1 mM EDTA in 10 mM Tris-HCl (pH 7.6) and incubated at 37°C for 10 min. 2 µl of 150 mM NaCl in 100 mM Tris-HCl (pH 7.5) were added to the nucleic acid solution followed by 2 µl of 1 mg/ml RNase A (from bovine pancreas). The 1 mg/ml RNase A solution was preheated at 95°C for 5 min to inactivate DNase. The nucleic acid solution was incubated with the RNase A solution for 2 h at 37°C. 30 µl of alkaline loading buffer (150 mM NaOH, 3 mM EDTA, 0.01% Ficoll 400, 0.075 % bromocresol green and 0.0125% xylene cyanol) was added to each sample and the microcentrifuge tubes incubated at 80°C for 5 min then cooled quickly using ice. Following centrifugation for 2 min, 10 µl of each sample were loaded onto a pre-prepared alkaline gel.

The alkaline gel was prepared from 40 ml of 0.8% agarose solution which was dissolved by heating. The solution was cooled to 60°C before adding 80 µl of EDTA (0.5 M, pH 8.0) and 400 µl of 5 M NaCl. The agarose solution was poured into a gel tank (Bio-Rad mini sub DNA cell) to a depth of 5 mm and left to set. The gel was pre-run in an alkaline buffer (30 mM NaOH and 1 mM EDTA (pH 8.0)) for 3 x 30 min periods (each time using fresh buffer) in the fridge at 50 V. Once the samples were loaded, the gel was run in fresh alkaline buffer at 24 mA (12 V) in the fridge overnight. Alternatively, the alkaline gel was run at 50 V in the fridge for 5 hours.

The gel was washed in a neutralising buffer (1.5 M NaCl and 1 M Tris-HCl (pH 7.8)) for 2 x 30 min periods and stained using ethidium bromide (1 µg/ml in 0.5 mM EDTA (pH 8.0),

5 mM Tris-HCl (pH 7.6)) also for 2 x 30 min periods. To remove unbound ethidium bromide, the gel was rinsed in water. The gels were photographed using a Polaroid MP 4 land camera (Polaroid 667 film) and a Flowgen transilluminator (312 nm). The intensity of ethidium bromide-stained DNA along each lane on the negative film of each gel was quantified using an image analyser (Aequitas 1A) and a light box. Readings were recorded along the empty lanes to quantify the background intensity.

2.2.4. **Field trials**

2.2.4.1. 1994 Field trials (1 - 3)

Three emergence trials were set up in spring 1994 (see Table 2.6). For each seedlot, four replicates of 250 seeds were sown. For field trials 1 and 2, the replicates were blocked by position in the field, with the seedlots being positioned randomly within each block (Appendices A1 and A2). For field trial 3, the blocks were defined by which one of the four holes in the Stanhay belt drill that each seedlot was sown through. For all trials, a seedling was counted as emerged once the hook of the hypocotyl was visible.

For trials 1 and 2, newly emerged seedlings were marked by a different coloured cocktail stick to indicate the day of emergence. If a seedling turned out to be a weed then the cocktail stick and the associated weed were removed. An electric fence was put round the plots of trial 1 and 2 to reduce seedling loss by vertebrate pests. An establishment count was made, once most seedlings had reached the 4-6 true leaf stage.

For trial 3, the number of seedlings per row was counted approximately every 4 days and once most seedlings had reached the 4-6 true leaf stage. The seedlings were not marked with cocktail sticks.

2.2.4.2. 1995 Field Trial (4)

The seeds of six seedlots (seedlot 2 experiment) were rubbed, sieved (3mm to 4.25 mm in diameter) and thiram-steeped. Eight blocks of 150 seeds per plot (3 rows) were sown using a Hege drill at Broom's Barn on 17 March 1995. The position of each seedlot was randomised

within each block (Appendix A3). The soil was a sandy loam (soil series Barrow) and the target seed spacing was 11 seeds per metre. An electric fence surrounded the field trial. Emergence was marked daily using cocktail sticks and the final establishment was counted at the 4-6 true leaf stage. The parts of the seedlings which were above ground were harvested the day after the final establishment count which was 56 days after sowing. The harvested seedlings for each plot were divided into those emerging early, those emerging in the middle of the counting period and those emerging late. The fresh weights were recorded and the dry weights taken after 48 h at 85°C.

TABLE 2.6 Summary of the field trials.

	Trial 1	Trial 2	Trial 3
aim	to assess the emergence of seedlots	to assess the emergence of steeped and advanced seeds	to assess the emergence of seedlots and density graded lots
location	IACR - Broom's Barn, Suffolk; sandy clay loam (soil series Barrow)	IACR - Broom's Barn, Suffolk; sandy clay loam (soil series Barrow)	Rides Farm, Gayton, Norfolk; sandy loam
seed	Cyrano A-J (10 lots) Marathon A-C (3 years) Matador A-D (4 countries)	Cyrano B,G,H (3 lots) Matador B,C,D (3 countries)	Rizor A-J (10 lots) Cyrano B,G,H (3 lots) Planet 1a,1b,2a,2b,3a, 3b,4a,4b (8 lots)
seed treatment	rubbing and thiram - steeping of all lots	rubbing of all lots, thiram-steeping or advancing of all lots	rubbing of Rizor and Cyrano lots and thiram-steeping of all lots
sowing	27 March 1994 using a Hege cone drill 9 seeds/m 1 plot = 3 rows	27 March 1994 using a Hege cone drill 9 seeds/m 1 plot = 3 rows	17 March 1994 using a Stanhay belt drill 16.7 seeds/m 1 plot = 1 row

FIGURE 2.2 The wavelength scan (200-600 nm) of calf thymus DNA standards (95 µl of 1 x TNE plus 5 µl of the DNA standard; a = 1000 µg/ml, b = 750 µg/ml, c = 500 µg/ml, d = 250 µg/ml DNA dissolved in 0.1 x TE).

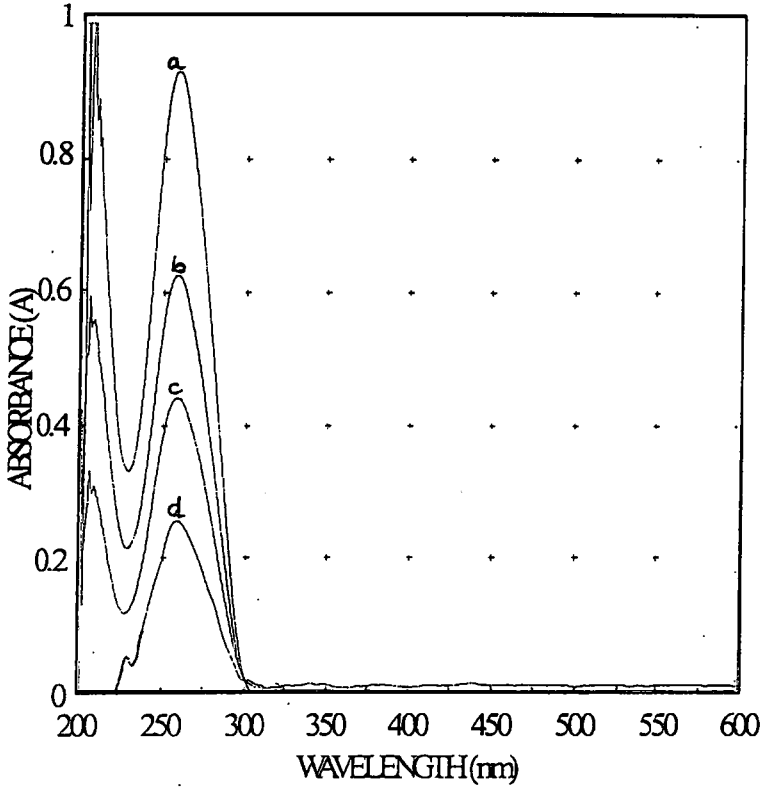


FIGURE 2.3 The wavelength scan (200-600 nm) of calf liver RNA standards (95 µl of 1 x TNE plus 5 µl of the RNA standard; a = 1000 µg/ml, b = 750 µg/ml, c = 500 µg/ml, d = 250 µg/ml and e = 100 µg/ml RNA dissolved in 0.1 x TE).

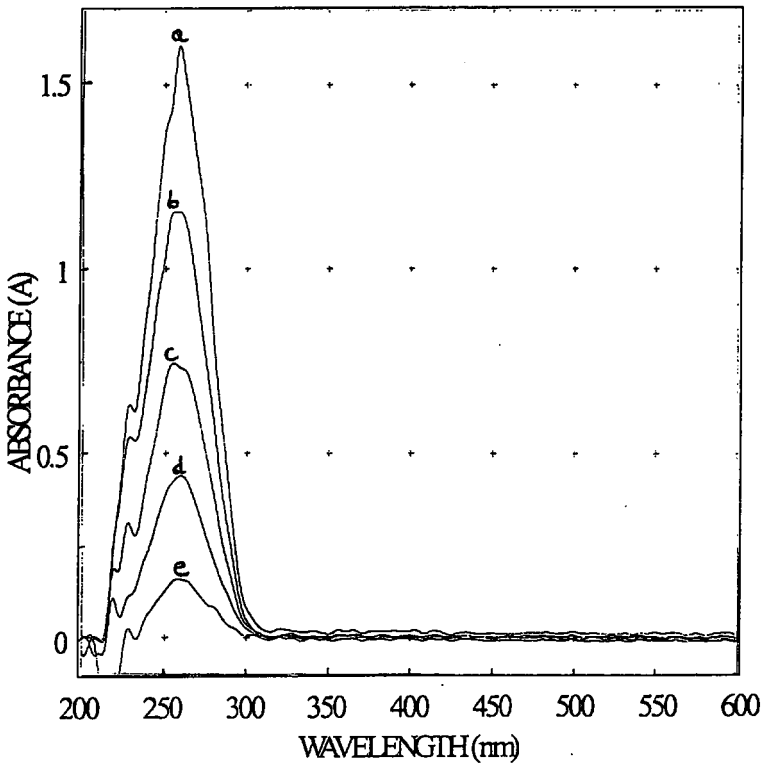


FIGURE 2.4 The wavelength scan (200-600 nm) of DNA and RNA (a = 90 µl of 1 x TNE, 5 µl of 250 µg/ml DNA standard and 5 µl of 250 µg/ml RNA standard, b = 95 µl of 1 x TNE plus 5 µl of 250 µg/ml RNA standard, c = 95 µl of TNE plus 5 µl of 250 µg/ml DNA standard).

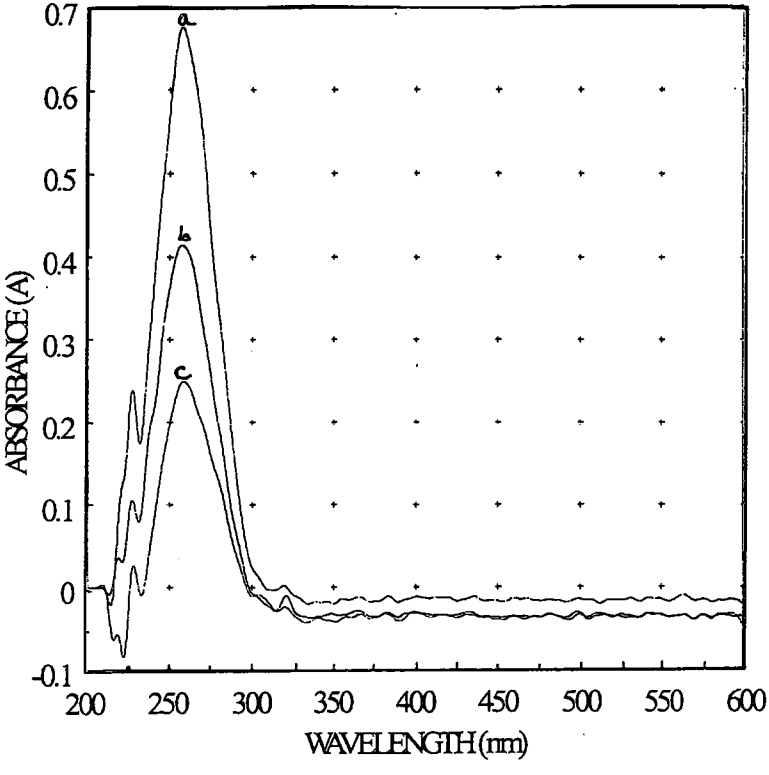


FIGURE 2.5 The effect on the UV-absorption spectra of including the seed coat during nucleic acid extraction and quantification. Solutions are 95 µl of 1 x TNE plus 5 µl nucleic acid solution extracted from either (a) 50 true seeds or (b) 10 fruits.

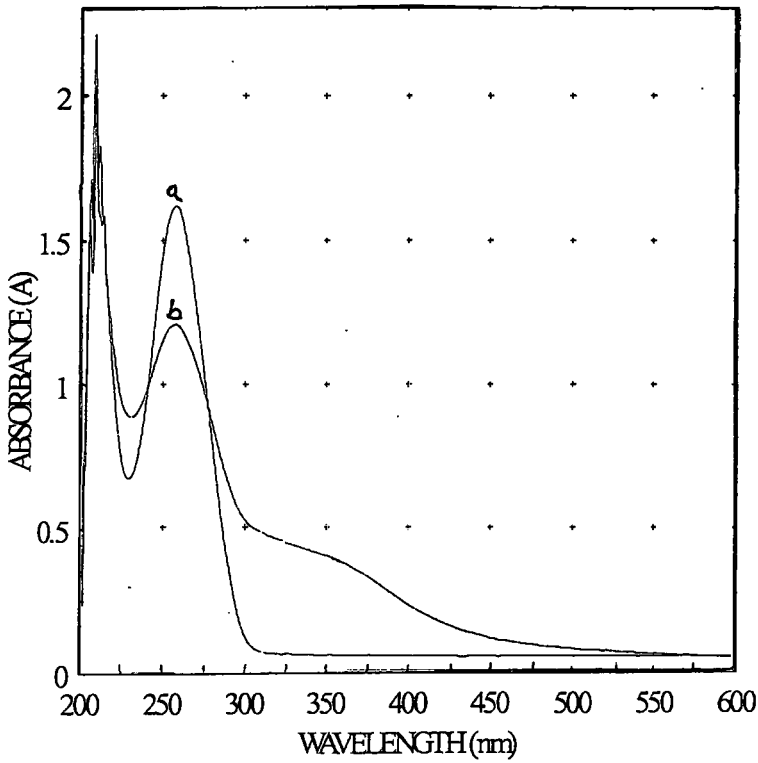


FIGURE 2.6 The effect of the number of true seeds on the quantity of nucleic acids extracted. Graph of wavelength scans of 95 µl of 1 x TNE plus 5 µl of nucleic acid solution extracted from (a) 50 true seeds, (b) 25 true seeds or (c) 10 true seeds.

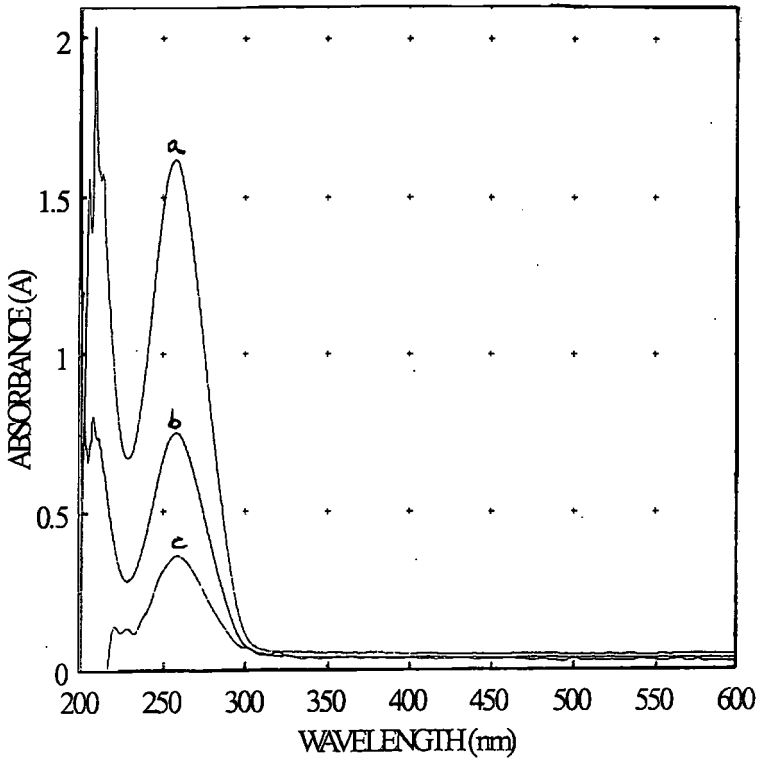


Figure 2.7 The emission wavelength scan of Hoechst dye and Hoechst dye bound to DNA using excitation at 360 nm (a = 995 µl working dye solution plus 5 µl of 500 µg/ml DNA standard, b = 995 µl of working dye solution).

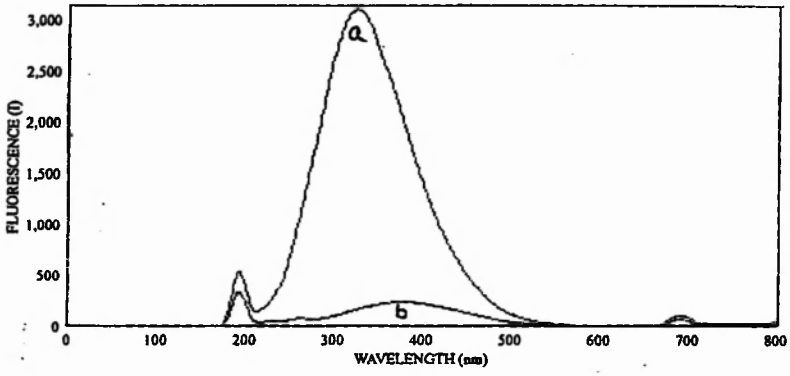


FIGURE 2.8 Calibration curve of calf thymus DNA standards on the fluorimeter (5 µl of DNA standard plus 995 µl of working dye solution, 3 replicates, $r = 0.995$ ($p < 0.01$)).

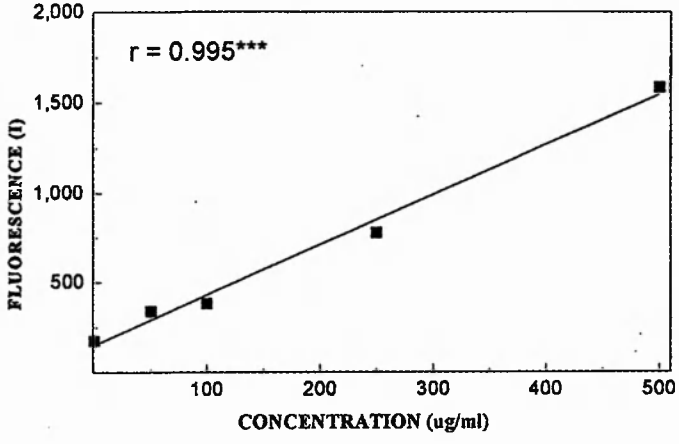
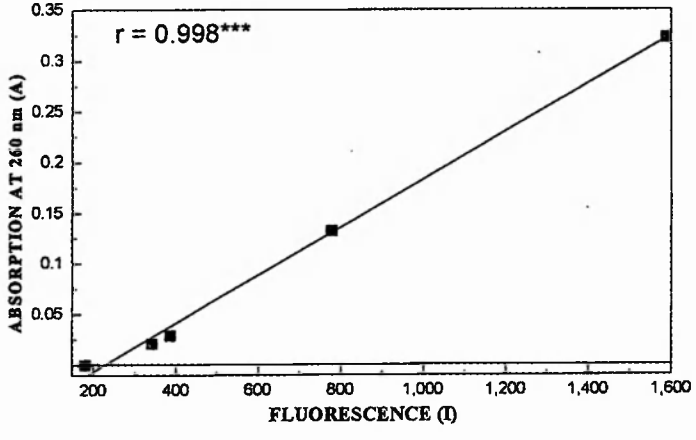


FIGURE 2.9 The calibration of calf thymus DNA standards measured on the UV/Vis spectrophotometer and the fluorimeter (0 µg/ml, 50 µg/ml, 100 µg/ml, 250 µg/ml and 500 µg/ml DNA standards).



3.1. LABORATORY ASSESSMENT OF VIGOUR

The germination characteristics (section 2.2.2.) and RNA/DNA ratios (section 2.2.3.) of each seedlot were determined. The germination means of each seedlot, established using a range of tests, were then compared with the means of the RNA/DNA ratios using correlation analysis. The statistical significance of the relationship between the performance of seedlots in the germination tests and their RNA/DNA ratios was determined by the calculation of the Pearson's correlation coefficient (referred to as 'correlation coefficient' in the text). The seedlots were also ranked in terms of performance in the germination tests and their RNA/DNA ratios. The degree of similarity in ranks between methods of seed assessment was calculated to give the Spearman's rank correlation coefficient. The conclusions from both analyses were similar, and in general the Pearson's correlation coefficients will be referred to here throughout. The statistical analyses are explained in more detail in Appendix B.

3.1.1. Seedlots 1 experiment

The means of the germination performance of each seedlot were calculated from three replicates (Appendix C1). The seedlots from cv. Rizor were assessed separately to the other seedlots. The seedlots were assessed by a range of germination tests and they will be described in the text as follows:

- the **cold stress test** at 9°C; the measurements recorded were time to 50% germination (T_{50}), mean time to germination (MGT) and mean time to the hypocotyls being longer than 2 cm (MHT)
- the **standard test at 15°C**; the measurements recorded were time to 50% germination (T_{50}), mean time to germination (MGT) and mean time to the hypocotyls being longer than 2 cm (MHT)
- the **standard test at 20°C**; the measurements recorded were the germination level (G%) and the percentage of seeds producing seedlings with hypocotyls longer than 2 cm (H%) ($G\%_{20C,dx}$ refers to the germination level on day x of the standard germination test at 20°C)

- the **cold sand test**; the measurements recorded were the emergence level with the count including abnormals (sand-1) and the count excluding abnormals (sand-2)
- the **wet stress test**; the measurements recorded were the germination level (G%) and the percentage of seeds producing seedlings with hypocotyls longer than 2 cm (H%) ($G\%_{wet,dx}$ refers to the germination level on day x of the wet stress test)

The RNA/DNA ratios for each seedlot (Appendix D1) were calculated in three different ways (section 2.2.3.3.1) and the means and standard errors determined. Both RNA/DNA ratio 2 and 3 were adjusted to take into account possible contaminants. The determination of RNA/DNA ratio 2 included the measurement of the absorbance of aromates at 280 nm on the spectrophotometer and RNA/DNA ratio 3 at 230 nm to correct for protein and phenol contamination.

3.1.1.1. Relationships between the RNA/DNA ratios and the germination test results

Table 3.1 shows the correlation between the performance of the seedlots in the germination tests and the RNA/DNA ratios. The seedlots of the cvs. Cyrano, Marathon and Matador were assessed together in a single experiment with three replicates. The majority of the germination test measurements correlated significantly ($p<0.05$) with the RNA/DNA ratios. The different methods of calculating the RNA/DNA ratios produced similar correlations with the germination test measurements. The germination test measurements which were not significantly correlated with the RNA/DNA ratios were: the MHT in the cold stress test and the germination test at 15°C, $H\%_{20C,d6}$ and $H\%_{wet,d4}$. The germination test results which were significantly ($p<0.05$) associated with the RNA/DNA ratios (determined by the Spearman's correlation coefficient) also correlated significantly with the RNA/DNA ratios using the Pearson's correlation analysis. This indicates that the germination tests which ranked the seedlots in a similar order to the RNA/DNA ratios also showed a linear relationship with the RNA/DNA ratios. There was one exception to this where the Spearman's rank correlation coefficient was significant ($p<0.05$) for RNA/DNA ratio 3 ranked against the performance of seedlots in the cold stress test (9°C-MHT) but the Pearson's correlation coefficient was not significant.

Examples of relationships with significant correlation coefficients include:

- The significant negative correlation ($p<0.001$) between RNA/DNA ratio 1 and T_{50} in the cold stress test (Figure 3.1). Those seedlots which germinated more quickly and had a smaller T_{50}, also had a larger RNA/DNA ratio. This significant correlation was evident in all three methods of calculating the RNA/DNA ratios. The seedlots from cv. Cyrano germinated more slowly than those of cvs. Marathon and Matador and also had smaller RNA/DNA ratios.
- The cv. Cyrano seedlots assessed in the germination test at 15°C also germinated more slowly than those lots from cvs. Matador and Marathon and had smaller RNA/DNA ratios (Figure 3.2).
- There was a positive significant ($p<0.001$) correlation between RNA/DNA ratio 3 and $G\%_{wet,d4}$ (Figure 3.3). Seedlots from cvs. Marathon and Matador had larger G% by day 4 of the test and had larger RNA/DNA ratios compared with those of cv. Cyrano.

Correlation coefficients between vigour assessments were also determined using the results for the seedlots of each cultivar in turn. The cultivar, Cyrano, had ten seedlots and none of the correlations between the germination test measurements and the RNA/DNA ratios were significant (Table 3.2). Data from the seedlots of cvs. Marathon and Matador would have too few degrees of freedom to carry out intra-cultivar correlations so that correlation coefficients were calculated for the seedlots of both cultivars together (Table 3.3). Measurements from the germination test (T_{50} and MHT) at 15°C correlated significantly with the RNA/DNA ratios. This significant correlation between the MHT values from the 15°C germination test and the RNA/DNA ratio contrasts with the lack of correlation when the data of the cv. Cyrano seedlots are included (Figure 3.4).

In the seedlots 1 experiment, there were ten seedlots of cv. Rizor. The correlation table is shown in Table 3.4. There were six significant correlations ($p<0.05$) between the RNA/DNA ratios (1-3) and G% at 20°C. Figure 3.5 shows the correlation between the RNA/DNA ratio 3 and the $G\%_{20C,d3}$. The cv. Rizor seedlots which had more seedlings on day 3 also had larger RNA/DNA ratios. In addition, when the seedlots were ranked, there was a significant ($p<0.05$) association between rate of germination in the cold stress test (T_{50}) and RNA/DNA ratio 3.

3.1.1.2. Relationships between the germination test results

The germination test measurements of each seedlot were analysed in order to establish which tests produced the most co-varying assessment of lot performance. When the seedlots of cvs. Cyrano, Marathon and Matador were assessed the following relationships were found:

- The cold stress test produced results which were significantly ($p<0.05$) correlated with most of the other germination test results (Table 3.5).
- The G% at 15°C correlated significantly with the other test results except for those from the cold sand test.
- The MHTs at 9°C and 15 °C did not correlate significantly with any of the other germination test results.
- The later counts of G% and H% at 20°C correlated significantly ($p<0.05$) with the other germination test results.
- The wet stress test measurements correlated significantly ($p<0.05$) with most of the other test measurements except for $H\%_{20C,d6}$ and the MHTs at 9°C and 15°C.

The correlation coefficients of the germination test results of the cv. Rizor seedlots are shown in Table 3.6. and are described as follows:

- The measurements from the germination tests at 9°C and at 15°C correlated significantly ($p<0.05$) with each other and with $H\%_{wet,d4}$ (correlation coefficient not shown in Table 3.6).
- The results from the cold sand test did not correlate significantly with the results from any other germination test except $G\%_{wet,d7}$, $G\%_{wet,d14}$ and $H\%_{wet,d14}$.
- The lot assessments, determined by the standard germination test at 20°C, only correlated significantly with certain measurements from the wet stress test (correlation coefficients not shown in Table 3.6).

3.1.2 Steeping-advancing experiment

3.1.2.1. Germination test results of treated seedlots

Advanced seeds performed better in the cold stress test than the thiram-steeped seeds when G%, MGT and MHT were measured (Appendix C2). An ANOVA of the data showed that advanced seeds had a significantly ($p<0.001$) larger G% than thiram-steeped seeds (Figure 3.6). The G% of treated seeds at 9°C was significantly ($p<0.01$) affected by which cv. Cyrano seedlot was sown. The interaction between the seedlot sown and seed treatment given was significant ($p<0.05$) because advancing did not improve the germination level of each lot to the same extent. However, in comparison with thiram-steeping, the advancement treatment produced a G% which was more uniform between the seedlots.

The advancement treatment, compared with thiram-steeping, also significantly ($p<0.001$) reduced the MGT at 9°C (Figure 3.7). There was a significant ($p<0.05$) difference between the MGTs of each seedlot except between advanced cv. Cyrano G and H seedlots and between steeped B and G seedlots. The decrease in MGT by the advancement treatment depended on the lot because the interaction between lot and treatment was significant ($p<0.05$).

The MHT was significantly ($p<0.001$) smaller for advanced seeds than for thiram-steeped seeds (Figure 3.8). This may be interpreted as the advancement treatment producing seeds from which hypocotyls grew earlier than from thiram-steeped seeds at 9°C. The factor, seedlot, was not a significant ($p<0.05$) factor which affected the MHT of treated seeds at 9°C. The interaction between the lot and treatment did not significantly ($p<0.05$) affect the MHT.

Treated seeds were also assessed using the standard germination test at 20°C (Appendix C2). The advancement treatment did not significantly ($p<0.05$) increase the G% at 20°C compared to thiram-steeping. On day 6 and day 14 of the germination test, the G% were significantly ($p<0.01$) affected by the cv. Cyrano seedlot sown.

3.1.2.2. The RNA/DNA ratios of treated seedlots

The RNA/DNA ratios for each treated lot are summarised in Appendix D2. For each of the

four calculations of the RNA/DNA ratio, treatment is a significant ($p<0.01$) factor. Therefore the RNA/DNA ratio of advanced seeds was significantly larger in comparison with the ratio of thiram-steeped seeds. Table 3.7 shows the percentage increase in RNA and DNA following the advancement treatment relative to the amounts from steeped seeds. The advancement treatment increased the amount of extractable DNA in comparison with the steeping treatment by 20-35% depending on the seedlot. The amount of extractable RNA was increased by 47-100% (data from RNA/DNA calculation 1). Seedlot B, which had the largest percentage increase in DNA following the advancement treatment, also had the largest percentage increase in RNA compared to the other two cv. Cyrano seedlots. The seedlot (lot H) which had the lowest increase in DNA (20%) also had the lowest increase in RNA (c.a. 50%) following the advancement treatment.

There were no significant ($p<0.05$) differences between the RNA/DNA ratios of each seedlot nor was the interaction between lot and treatment significant.

3.1.2.3. Relationships between the RNA/DNA ratios and the germination test results

The means of the germination test measurement for each cv. Cyrano seedlot were compared with the means of the RNA/DNA ratios of each lot using correlation analysis (Table 3.8). The RNA/DNA ratios (all four calculations) correlated significantly ($p<0.05$) with the MGT and MHT in the cold stress test but not with the germination counts at 20°C. In Figure 3.9, the RNA/DNA ratios 1 of the steeped and advanced seeds correlated significantly ($p<0.01$) with MGT at 9°C. The advancement treatment, which decreased the MGT, also increased the RNA/DNA ratio of each seedlot.

3.1.3. Seedlots 2 experiment

3.1.3.1. Relationships between the RNA/DNA ratios and the germination test results

The germination test results of seedlots 2 experiment are summarised in Appendix C3. The RNA/DNA ratios for each lot (Appendix D3) were determined using ten different calculations (section 2.2.3.3.2.). Both sets of vigour assessment data were analysed to see how closely the RNA/DNA ratios of each seedlot correlated with the germination test measurements. None

of the correlation coefficients in Table 3.9 was significant ($p<0.05$) nor were the Spearman's rank correlation coefficients. This may be due to the small number of degrees of freedom. However, two calculations, RNA/DNA ratio 7 and 8, produced, in general, larger correlation coefficients than the others. These calculations included an estimate of contamination by measuring absorbance at 230 nm and also utilised a calibration curve generated using DNA standards measured on the UV spectrophotometer and the fluorimeter (Figure 2.9). Examples of relationships between the RNA/DNA ratios and germination test results are as follows:

- In Figure 3.10, the correlation coefficient is 0.65 and the lots which had a larger G% on day 2 of the wet stress test also had a larger RNA/DNA ratio 1.
- The negative correlation of MHT at 9°C with the RNA/DNA ratio 9 indicates that seedlots which produced hypocotyls larger than 2 cm more quickly, also had a larger RNA/DNA ratios except for cv. Cyrano G seedlot (Figure 3.11). Contrary to the general negative correlation, the two cv. Cyrano lots had small RNA/DNA ratios and smaller MHTs ie. the seeds produced hypocotyls larger than 2 cm more quickly.
- The same negative correlation is seen in Figure 3.12. The seedlots with small RNA/DNA ratios 6 germinated more slowly (except for the cv. Rizor F seedlot which had a larger RNA/DNA ratio but germinated more slowly at 9°C than would be expected).

3.1.3.2. Relationships between germination test results

The germination test measurements of each seedlot were investigated using correlation analysis in order to establish which germination tests produced the most co-varying assessment results:

- The measurements from the cold stress test did not correlate significantly with any of the measurements from the standard germination test at 20°C (Table 3.10). The results from the cold stress test only correlated significantly ($p<0.05$) with one wet stress test measurement; H% on day 4. Therefore the measurements determined from the cold stress test do not tend to assess the vigour of the seedlots in the same way as the other germination tests.
- In general, the seedlot assessments made at 20°C correlated significantly ($p<0.05$) with

the wet stress test measurements also conducted at 20°C but with a greater water substrate saturation level.

- The cold sand test also correlated significantly ($p<0.05$) with most of the wet stress test measurements, $H\%_{20C,d7}$ and $H\%_{20C,d15}$.

3.2. FIELD AND LABORATORY ASSESSMENT OF VIGOUR

The means of the vigour assessment measurements made in the laboratory by germination tests and RNA/DNA ratios for each seedlot were compared with their field performance using correlation analysis.

3.2.1. Field trial 1

3.2.1.1. Field trial results

Trial 1 assessed the performance of seedlots from seedlots 1 experiment in the field except the seedlots of cv. Rizor which were assessed separately in field trial 3. The daily emergence of seedlings in the field was summed to calculate the sum% (Appendix E1). The establishment count (establishment%) was made at the 4-6 true leaf stage and was smaller than the sum% because of seedling death after emergence. The rate of seedling emergence in the field was estimated by calculating mean time to emergence (MET), time to 50% emergence of seeds sown (T_{50}), time to 50% of emerged seedlings (T_{50}*) and time to 30% emergence (T_{30}).

The field trial data were analysed using ANOVA (Appendix B). When the data were blocked by position in the field, the cultivar and seedlot were significant factors ($p<0.001$) which affected the sum%, establishment%, MET, T_{50}* and T_{30}. An analysis of variance was not carried out on the T_{50} due to some of the plots not reaching 50% emergence of the seeds sown. To analyse whether there were greater differences between the cultivars rather than between the lots, an F-ratio was calculated by dividing the mean square of the factor, cultivar, by the mean square of the factor, lot. There were significantly ($p<0.05$) greater inter-cultivar differences than intra-cultivar differences for sum%, establishment% and T_{30} but not for MET and T_{50}*.

3.2.1.2. Relationships between the RNA/DNA ratios and the field trial measurements

Correlation coefficients of the RNA/DNA ratios of the seedlots of cvs. Cyrano, Marathon and Matador with the field trial results were determined (Table 3.11). All three RNA/DNA ratio calculations (1, 2 and 3) correlated significantly ($p<0.05$) with sum%, establishment%, MET and T_{30}. RNA/DNA 3 also correlated significantly ($p<0.05$) with T_{50} and T_{50}* and RNA/DNA 2 with T_{50}*. RNA/DNA ratio calculations 2 and 3 both include in their calculations estimates of protein contamination. Examples of relationships with significant correlation coefficients include:

- RNA/DNA ratio 3 is significantly correlated with the establishment count for each seedlot (Figure 3.13). The seedlots with the highest establishment also had the largest RNA/DNA ratios.
- A negative correlation is seen between the RNA/DNA ratios and MET (Figure 3.14). This is because the seedlots which have the fastest emergence, and therefore smaller METs, also have larger RNA/DNA ratios.
- A significant negative correlation is also seen between the RNA/DNA ratios 2 and T_{30} in the field (Figure 3.15).
- Only RNA/DNA ratio 3 correlated significantly ($p<0.05$) with field T_{50}. In addition, the Spearman's rank correlation coefficients were significant ($p<0.05$) for RNA/DNA ratio 1 and RNA/DNA ratio 2 with field T_{50}.
- RNA/DNA ratio 2 and RNA/DNA ratio 3 correlated significantly with T_{50}*.

If the vigour assessments of only cv. Cyrano seedlots are compared, then there are no significant correlations between the RNA/DNA ratios and the field trial measurements (Table 3.12). This is also the case when the correlation coefficients of cvs. Marathon and Matador seedlots are determined (Table 3.13).

3.2.1.3. Relationships between the germination test results and the field trial measurements

In order to compare the usefulness of laboratory germination tests in predicting the field performance of seedlots, a correlation analysis of the germination test results of cvs. Cyrano, Marathon and Matador seedlots with field trial 1 results were made (Table 3.14):

- The T_{50} and MGT in the standard germination tests at 9°C and 15°C correlated significantly ($p<0.05$) with lot performance assessed in the field by the different measurements. Figure 3.16 shows the significant ($p<0.001$) negative correlation between establishment in the field and T_{50} at 9°C.
- The MHT determined from the germination test at 9°C did not correlate significantly with any of the field trial assessments.
- Most of the germination and hypocotyl counts from the germination test at 20°C, the cold sand test and the wet stress test correlated significantly ($p<0.05$) with sum%, establishment%, T_{50} and T_{30} determined in the field trial.
- The later germination counts at 20°C, germination in the cold sand test and the counts of the seedlots in the wet stress test did not correlate significantly with MET and T_{50}*.

If the germination test results and field performance of the cv. Cyrano seedlots are analysed without cvs. Marathon and Matador (Table 3.15), then few of the correlations between the germination test and field trial measurements are significant. The significant correlations were between the $H\%_{wet,d4}$ and $H\%_{20C,d6}$ with field performance. When the performance of the seedlots from cvs. Marathon and Matador in germination tests were compared with field performance (Table 3.16), there were some significant correlations between laboratory performance and rate of emergence in the field:

- Rate of germination in the cold stress test, rate of germination in the standard germination test at 15°C and $G\%_{20C,d3}$ correlated significantly ($p<0.05$) with rate of emergence in the field.
- $H\%_{20C,d14}$ and germination in the sand test (2) showed significant positive correlations with MET and T_{50}* in the field instead of the expected negative correlations.

3.2.2. Field trial 2

3.2.2.1. Field trial results

Field trial 2 assessed the field performance of three cv. Cyrano seedlots and three cv. Matador seedlots which had been thiram-steeped or given an advancement treatment (Appendix E2). The field trial data were analysed using the ANOVA. When the data were blocked for position

in the field, there was a significant ($p<0.001$) difference between the cv. Cyrano seedlots and the cv. Matador seedlots in terms of sum% and establishment%. There were no significant ($p<0.05$) differences between the METs of the cultivars. There were significantly ($p<0.05$) larger differences between cv. Cyrano and cv. Matador than between the lots within each cultivar for sum% and establishment% but not for MET.

Sum%, establishment% and MET of the advanced seeds were significantly ($p<0.001$) different to those of the steeped seeds. The advancement treatment in comparison with thiram-steeping improved the sum% (Figure 3.17), the establishment% (Figure 3.18) and the MET (Figure 3.19) in the field. The interaction between cultivar and seed treatment was not a significant ($p<0.05$) factor which affected the sum%, establishment% and MET. The interaction of seedlot with seed treatment was a significant ($p<0.05$) factor which affected the sum%, establishment% and MET in the field. This means that the effect of the seed treatment on field performance was dependent upon which seedlot was treated, ie. the advancement treatment did not improve the field performance of each seedlot to the same extent.

3.2.2.2. Relationships between the RNA/DNA ratios and field trial measurements

The RNA/DNA ratios determined for the steeped or advanced cv. Cyrano seedlots were compared with the field performance measurements in order to assess the accuracy of the RNA/DNA ratio for predicting field performance. All four calculations of the RNA/DNA ratio correlated significantly ($p<0.01$) with the field performance of the thiram-steeped or advanced cv. Cyrano lots (Table 3.17). Figure 3.20 shows the significant ($p<0.01$) correlation between RNA/DNA ratio 1 and establishment in the field. The advanced seeds, which showed better field establishment than thiram-steeped seeds, also had a larger RNA/DNA ratio. The significant negative correlation in Figure 3.21 is due to advanced seeds having a smaller MET and a larger RNA/DNA than steeped seeds.

3.2.2.3. Relationships between the germination test results and field trial measurements

The assessments of the treated cv. Cyrano lots by the germination tests at 9°C and 20°C and the field measurements were analysed by correlation analysis in order to compare the accuracy of the germination tests as predictors of field performance (Table 3.18):

- The measurements at 9°C correlated significantly ($p<0.05$) with the field performance measurements. In Figure 3.22, advanced seeds had a higher establishment count in the field and germinated more quickly at 9°C than the steeped seeds.
- The germination counts at 20°C did not correlate significantly ($p<0.05$) with sum%, establishment% and MET determined in the field. The germination test at 20°C did not reflect the improvement in seed performance in the field facilitated by the advancement treatment (Figure 3.23).

3.2.3. Field trial 3

Trial 3 assessed the performance of cv. Rizor seedlots and cv. Planet lots which had been rubbed and graded to different extents. Three of the cv. Cyrano seedlots were also included in order to compare their field performance in the three different field trials.

3.2.3.1. The field trial results of seedlots from cv. Planet

The field performance of the cv. Planet lots are summarised in Appendix E3. The data were analysed using ANOVA. Seeds which had a high density had a significantly ($p<0.001$) higher establishment percentage in the field than those seeds of medium density (when the data were blocked for the hole in the drill that the seedlot was sown through). The denser seeds also emerged more quickly than the seeds of medium density ie. T_{50} was significantly less (Figure 3.24). The degree of rubbing, was a significant factor ($p<0.05$) which affected the MET, T_{50}, T_{50}* and T_{30} in the field. Seedlots which had been rubbed harder emerged in the field more quickly, for example, T_{50} was less for seeds which had been rubbed more (Figure 3.24).

3.2.3.2. The field trial results of cv. Rizor seedlots and cv. Cyrano seedlots

The field trial results of the ten cv. Rizor seedlots and the three cv. Cyrano seedlots (Appendix E4) were analysed using ANOVA. There was a significant ($p<0.05$) difference between the seedlots of cv. Rizor and those of cv. Cyrano in terms of establishment, MET and T_{50}* if the data were blocked for the sowing position in the drill. The seedlot sown did not significantly ($p<0.05$) affect field performance except for T_{50}*. There were significantly ($p<0.05$) larger

inter-cultivar differences than intra-cultivar differences for establishment, MET and T_{50}* but not for T_{30}.

The three cv. Cyrano seedlots, B, G and H, were sown in all three field trials (Appendix E5). Seedlot B of cv. Cyrano performed the worst in all the trials for the different measurements except in trial 3 for T_{50}. The cv. Cyrano G seedlot performed better in the field than the other two seedlots on most occasions although Cyrano H was marginally the better of the three in terms of establishment in trial 2 and for MET and T_{30} in trial 3.

3.2.3.3. Relationships between the RNA/DNA ratios, germination test results and field trial measurements of cv. Rizor seedlots

The correlation coefficients were determined for the field measurements of the cv. Rizor seedlots and the RNA/DNA ratios (Table 3.19). None of the correlations were significant ($p<0.05$), for example, Figure 3.25 shows the negative correlation between MET in the field and the RNA/DNA ratio 1. Spearman's rank correlation coefficients were significant ($p<0.05$) for RNA/DNA ratio 2 with field MET and T_{50}.

A correlation analysis was made of the laboratory germination test results of the cv. Rizor seedlots with the field trial measurements (Table 3.20):

- The results from the germination tests at 9°C and 15°C did not correlate significantly ($p<0.05$) with the field trial measurements.
- For the germination test at 20°C, only $H\%_{20C,d6}$ correlated significantly ($p<0.05$) with the field trial measurements of MET and T_{50} (Figure 3.26).
- The significant correlations ($p<0.05$) of field T_{50}* with the cold sand test, $H\%_{wet,d14}$ and $G\%_{wet,d14}$ would be expected to be negative correlations rather than positive correlations. In Figure 3.27, the cv. Rizor seedlots which germinated more quickly in the field had a lower G% in the cold sand test.
- The significant ($p<0.05$) correlation between the cold sand test and field T_{30} was also a positive correlation rather than an expected negative correlation.

3.2.4. Field trial 4

3.2.4.1. The field trial results

The field trial results for seedlots 2 experiment are summarised in Appendix E6. When the data were analysed using ANOVA, it was found that the factor, seedlot, significantly ($p<0.01$) affected sum%, establishment% and T_{50}* in the field. The position of the blocks in the field trial did not significantly ($p<0.05$) affect emergence in the field.

The emerged seedlings were harvested once it was decided that final establishment had been reached. The seedlings of each plot were divided into three groups (a, b and c) based on their time of emergence. The fresh weights per plot and per plant in each plot are summarised in Appendix E7. The fresh weights of seedlings per plot that emerged early on (fresh weight a) and in the middle (fresh weight b) of the counting period were significantly ($p<0.01$) affected by which seedlot was sown. The fresh weights per plant of seedlings which emerged in the middle of the counting period were also significantly ($p<0.01$) affected by the seedlot which was sown in each plot. The position of the blocks in the field trial was not a significant factor ($p<0.05$) which affected the fresh weight of the harvested seedlings except for the earlier emerging seedlings (fresh weight a).

The dry weights were approximately 10% of the fresh weights (Appendix E8). The seedlot sown significantly ($p<0.01$) affected the same weight measurements as those of the fresh weights. The position of the blocks in the field trial did not significantly ($p<0.05$) affect the dry weights except for the dry weight per plant of those seedlings which emerged in the middle of the counting period (dry weight/plant b).

3.2.4.2. Relationships between the RNA/DNA ratios and field emergence measurements

Correlation coefficients were determined for the RNA/DNA ratios and the field trial measurements of each seedlot (Table 3.21). None of the correlations was significant ($p<0.05$). Figure 3.28 shows the negative correlation between the RNA/DNA ratio 3 and establishment in the field. It was expected that the correlation values would have been positive because the seedlots with the better establishment counts would have larger RNA/DNA ratios (as seen for

seedlots 1 experiment). Negative, instead of the expected positive, correlation coefficients were also seen between RNA/DNA ratio 3 and sum% and RNA/DNA ratio 7 and 8 with establishment% and sum%. The correlation between the RNA/DNA ratios 7 and MET was a negative correlation which may be explained by the seedlots which emerged more quickly in the field having larger RNA/DNA ratios (Figure 3.29).

3.2.4.3. Relationships between the RNA/DNA ratios and harvested seedling weights

A correlation analysis was used to compare the RNA/DNA ratios with the fresh and dry weights of the harvested seedlings (Table 3.22). None of the correlations was significant ($p<0.05$) except for RNA/DNA ratio 3 correlating significantly with total fresh weight and total dry weight per plot (Figure 3.30). The negative correlation in Figure 3.30 is unexpected in that the seedlots with the larger RNA/DNA ratios produced the smallest dry weight of harvested seedlings per plot. This significant negative correlation was also seen when the seedlots were ranked to produce Spearman's rank correlation coefficients for the measurements as follows:

- RNA/DNA ratios 1, 3, 4, 5, 6, 9 and 10 with fresh weight/plant b.
- RNA/DNA ratios 5, 6, 9 and 10 for dry weight/plant b.
- RNA/DNA ratio 3 with total fresh weight and total dry weight.

3.2.4.4. Relationships between the harvested seedling weights and field emergence measurements

Correlation coefficients were determined for the seedling weights with the emergence measurements from the field trial (Table 3.23):

- Sum% correlated significantly ($p<0.05$) with the fresh and dry weights of late emerging seedlings (period c) and total dry weights of each plot.
- The establishment count correlated significantly ($p<0.05$) with the total fresh and dry weights for each plot. In Figure 3.31, as would be expected, the plots with more seedlings had larger total weights of harvested seedlings.
- The dry weights of the early emerging seedlings did not follow the same trend of

increasing with increasing establishment unlike the other dry weights.

- MET correlated significantly ($p<0.05$) with fresh and dry weights of early emerging seedlings (period a) and those seedlings emerging in the middle of the counting period (period b).
- MET also correlated significantly with fresh and dry weight per plant b.
- T_{30} emergence was significantly ($p<0.05$) correlated with fresh and dry weights per plant of early and late emerging seedlings. Figure 3.32 shows that the slower emerging seedlings had larger dry weights per plant so that on average each seedling was bigger.
- T_{50}* correlated significantly with the fresh and dry weights of early emerging seedlings (period a) and the fresh and dry weights per plant of seedlings which emerged in the middle of the counting period (period b). T_{50}* also correlated significantly ($p<0.05$) with the fresh weight of seedlings emerging in period b.
- The Spearman's rank correlation coefficients were significant ($p<0.05$) for the same measurements as the previous correlations but establishment ranked against fresh weight b and c and dry weight b and c were also significant ($p<0.05$).

3.2.4.5. Relationships between the germination test results and field emergence measurements

A correlation analysis was made of the field trial results of seedlots 2 experiment with the germination test results in order to assess any similarities between the results (Table 3.24):

- Sum% correlated significantly ($p<0.05$) with $H\%_{20C,d7}$ and $H\%_{20C,d15}$.
- The field establishment count did not correlate signficantly ($p<0.05$) with any of the germination test results.
- The MET in the field correlated significantly with the MHT at 9°C and $H\%_{wet,d4}$.
- T_{30} in the field correlated significantly ($p<0.05$) with T_{50} and T_{30} in the cold stress test (Figure 3.33).
- T_{50}* in the field was significantly correlated with T_{50}, T_{50}* and MGT in the cold stress test and $H\%_{wet,d4}$.

3.2.4.6. Relationships between the germination test results and harvested seedling weights

The germination test results were also compared with the seedling weights from the field trial

to investigate how well the germination tests predicted seedling performance in the field. The correlation coefficients for the fresh seedling weights and the dry seedling weights with the germination test results are shown in Table 3.25 and Table 3.36 respectively:

- The seedling weights (fresh and dry) of early emerging seedlings (period a) and average seedling weight for seedlings emerging late in the counting period (fresh and dry/plant c) correlated significantly ($p<0.05$) with most of the measurements from the cold stress test.
- There was an unexpected negative correlation between the cold stress test measurements and total seedling weight in a plot and average seedling weight of those plants emerging in period b.
- Late emerging seedlings (fresh and dry c) correlated significantly with some of the measurements from the wet stress test, the cold sand test and the standard germination test at 20°C.

3.3. NUCLEIC ACID SYNTHESIS AND INTEGRITY IN RELATION TO VIGOUR

3.3.1. **Factors affecting the RNA/DNA ratio**

3.3.1.1. Fruit size

Fruit size was studied using cv. Zulu seeds which had been graded into ten 0.25 mm fractions. Previous studies on size fractions of cv. Zulu had demonstrated a positive correlation between fruit size, rate of emergence, early seedling weight, final root yield and sugar yield (Thomas and Yallop 1994). To study the effect of fruit size on the RNA/DNA ratio, fruits from grade 1 (fruits less than 3.5 mm in diameter), 3 (3.75 mm to 4 mm), 5 (4.25 mm to 4.5 mm), 7 (4.75 mm to 5 mm), 9 (5.25 to 5.5 mm) and 10 (fruits more than 5.5. mm in diameter) were analysed. The means of six replicates of the RNA/DNA ratios are shown in Appendix D4. Fruit size had a significant ($p<0.05$) affect on the RNA/DNA ratio. The larger diameter fruits have, in general, higher RNA/DNA ratios except for the largest sizes, grades 9 and 10 (Figure 3.34). The amount of extractable DNA and extractable RNA also increased with fruit size except for the largest sizes (grades 9 and 10) which had smaller amounts of nucleic acids than grade 7 (Appendix D5).

Correlation coefficients were determined for the RNA/DNA ratios for each fruit size against field trial data and seed size measurements provided by T.H. Thomas and S.J. Yallop (Table 3.27). The RNA/DNA ratios (1, 2 and 4) correlated significantly ($p<0.05$) with the mean true seed weight and the weight of 1000 fruits. The same RNA/DNA ratios were also significantly correlated with MET and T_{50} in the field. In Figure 3.35, the larger fruits germinated more quickly and had larger RNA/DNA ratios than the smaller fruits. The seedlings of each fruit size were harvested and weighed. Some of these measurements correlated significantly with the RNA/DNA ratios (calculations 1 to 4) including the dry weight per plant.

3.3.1.2. Ploidy

To investigate the effect of ploidy on the RNA/DNA ratio, three pairs of commercial seed bulks were selected so that the diploid and triploid in each pair had similar germination test data at 20°C (pairs 1, 2 and 3). An additional pair of triploid bulks which had different germination test data (pair 4) were also assessed using the RNA/DNA ratio (Appendix D6). When the data from pairs 1, 2 and 3 were analysed using ANOVA, it was found that the pair and the ploidy level were not factors which significantly affected the RNA/DNA ratios. There was no trend of one ploidy level having a higher RNA/DNA ratio than the other ploidy (Figure 3.36) within the material used. In general, the triploid of each pair had a larger amount of extractable DNA than the diploid with the same germination performance. The triploid bulks also had more extractable RNA than the diploid in each pair (Appendix D7).

3.3.1.3. Seed treatments

3.3.1.3.1. *Seed treatments 1*

Three cv. Cyrano seedlots were thiram-steeped, advanced or left untreated in order to study the effect of seed treatments on the RNA/DNA ratio (Appendix D8). The RNA/DNA ratios of the advanced and thiram-steeped seeds have already been analysed in conjunction with the germination test data (section 3.1.2.) and the field trial 2 data (section 3.2.2.). Using ANOVA, it was found that the seed treatment significantly ($p<0.05$) affected the RNA/DNA ratios. The advancement treatment significantly ($p<0.05$) increased the RNA/DNA ratio in comparison to untreated and thiram-steeped seeds (Figure 3.37). The thiram-steeped seeds had smaller

RNA/DNA ratios than untreated seeds. Following the thiram-steep, the amount of DNA increased by 7-15% while the amount of extractable RNA decreased or only increased slightly (Appendix D9).

3.3.1.3.2. *Seed treatments 2*

The effect of artificially ageing untreated seeds and seeds which had already undergone an advancement treatment was investigated using the standard germination test at 20°C and the RNA/DNA ratio. The same cv. Cyrano seedlot, H, was used for all the treatments. The ageing process reduced the G% at 20°C of untreated and advanced seeds (Appendix C4). The effect of the treatments on the RNA/DNA ratios are shown in Appendix D10. An analysis of variance showed that the factor, treatment, significantly ($p<0.05$) affected the RNA/DNA ratio. Advanced seeds had the highest RNA/DNA ratio followed by advanced seeds which had been artificially aged (Figure 3.38). The untreated seed and the aged seeds had the smallest RNA/DNA ratios. The RNA/DNA ratios of the advanced and advanced then aged seedlots were significantly ($p<0.05$) different to the untreated and aged seedlots.

3.3.2. DNA synthesis

Allium cepa root tip nuclei were used as the standard for the amount of DNA per nucleus at the 2C stage of the cell-cycle. Nuclei which had a greater intensity of stain, in general, were smaller in size (Figure 3.39). The product of the intensity and detected area of each nucleus was used to determine the DNA content per nucleus. The percentages of nuclei with each of the DNA contents were calculated for each source of nuclei in order to determine the stage in the cell-cycle. Although the material used was from a triploid cultivar (cv. Cyrano) the terms 2C and 4C will be used to refer to the DNA content of nuclei before and after DNA synthesis irrespective of the ploidy level (Bennett and Leitch 1995).

At the completion of maturation in most plant embryos, the cycle arrests in the G_1 phase with a 2C DNA content (Baker and Bradford 1995). The DNA contents of the cotyledon leaf nuclei and embryo root tip nuclei from the same seedlot (cv. Cyrano G) were compared (Figure 3.40). Both tissues contained a high proportion of nuclei with 2C DNA levels. The percentage of cells with a 4C DNA content was calculated by doubling the DNA content at the 2C peak

and then counting the number of cells with the 4C DNA content (Table 3.28). The leaf and root tip cells had a low level of 4C nuclei (2%).

The percentage of nuclei with each DNA content was calculated for root tips from the embryos of seeds which had been treated by the advancement treatment and/or artificial ageing. The advancement treatment increased the percentage of nuclei with a 4C DNA content from 2% to 14% with an additional 10% of nuclei having a DNA content greater than 4C. Therefore there were fewer nuclei with the smaller DNA contents (Figure 3.41). Artificially ageing the seeds slightly increased the percentage of nuclei with smaller DNA contents compared to untreated seeds and advanced seeds. Ageing the seeds which had already been advanced, decreased the percentage of nuclei with a DNA content of 4C and above from 24% to 16%.

Three cv. Cyrano seedlots were selected for their different vigour levels. The seedlot, cv. Cyrano H germinated more quickly in a test at 9°C, showed better germination at 20°C and in the wet stress test and had larger RNA/DNA ratios (Appendices C1 and D1). The seedlot, cv. Cyrano B performed better than cv. Cyrano G so that the three seedlots could be ranked in terms of decreasing vigour in the order: H, B then G. Figure 3.42 shows the DNA contents of the nuclei from each cv. Cyrano seedlot. The cv. Cyrano H root tip cells had a slightly higher proportion of smaller DNA contents than those of cv. Cyrano G and B although 11% of cv. Cyrano H cells had 4C nuclei in comparison with 5% (Cyrano B) and 2% (Cyrano G).

The amount of DNA per cell can be estimated using the standard onion 2C value of 33.5 pg of DNA per nucleus (Bennett and Smith 1976). From Figure 3.40 it can be seen that the 2C peak approximates at the DNA content of 107500 arbitary units. For each tissue, the DNA content at the 2C peak was estimated using the graphs in Figure 3.40 and 3.42 and the amount of DNA calculated in Table 3.29. Bennett and Smith (1976) estimated the 2C DNA content of *B. vulgaris* as 2.5 pg per nucleus which is of a similar magnitude to the values in Table 3.29. Although there was some variation seen in the estimates of 2C DNA contents per nucleus from the leaf and root tip material, the range was relatively small (0.94 to 2.26 pg). One possible explanation is that an alternative method of quantification was used instead of the more standard microdensitometry and flow cytometry.

3.3.3. DNA repair

To establish whether DNA repair occurred in sugar-beet embryos during imbibition, the DNA of cv. Cyrano C embryos was damaged by γ-irradiation. Figure 3.43 shows the extent of DNA fragmentation when the samples were run on an alkaline gel. When the intensity of staining for each sample was measured along each lane, a DNA fragmentation profile of each treatment was generated (Figure 3.44). The higher molecular weight DNA was found closest to the origin and the molecular weight decreased progressively down the gel. γ-Irradiation of the embryos increased the fragmentation of the DNA which was seen by an increase in smaller molecular weight DNA compared to unirradiated embryos. The fragmentation profile of the control embryos had the highest intensity reading at approximately 20 units from the origin whereas the irradiated embryos had a DNA profile which peaked at approximately 60 units. If the irradiated embryos were imbibed for two hours, then the DNA profile moved to a higher molecular weight, with the maximum intensity at around 20 units from the origin.

The intensity of ethidium bromide-stained DNA was calculated for four segments down the alkaline gel for each lane. In Table 3.30, segment A includes the measurements nearest to the origin which corresponds to the highest molecular weight DNA fragments. The smaller DNA fragments migrate further down the gel so that segment D includes the smallest molecular weight fragments. The percentage of the total intensity readings for each segment was calculated. The DNA from the control embryos had nearly 20% of the total fragments in the highest molecular weight segment A whereas the irradiated embryos had just over 10%. The irradiated embryos which were subsequently imbibed for two hours had 19% of the total fragments in the highest molecular weight segment. This demonstrated that the two hour imbibition period following irradiation facilitated a joining of the smaller molecular weight fragments to produce more larger molecular weight fragments; this process being DNA repair.

Two cv. Rizor seedlots (B and F) were selected for performing differently in a range of germination tests but having a similar viability at 20°C (Appendix C1). Four replicates of embryos were used to compare the extent of DNA repair during a two hour imbibition at 24°C following γ-irradiation. Samples were run on an alkaline gel overnight and for five hours. Tables 3.31 and 3.32 show the intensity of staining in each segment of the gels. Most of the irradiated embryos, regardless of their vigour level, had a lower percentage of stained DNA

in segment A which indicated that the irradiation treatment reduced the amount of H.M.W. DNA in comparison with untreated embryos. Following imbibition at 24°C, the percentage of stained DNA in segment A increased in comparison with the irradiated embryos. This increase in segment A following imbibition can be used to quantify DNA repair. The level of DNA damage following irradiation needs to be taken into account so that the percentage improvement following imbibition can be calculated as:

$$\%\text{improvement} = \frac{(\%\text{intensity in segment A})_{\text{imbibed}} - (\%\text{intensity in segment A})_{\text{irradiated}}}{(\%\text{intensity in segment A})_{\text{irradiated}}}$$

There were no consistent differences found between the percentage improvement during imbibition following the irradiation treatment of high (cv. Rizor B) and low vigour (cv. Rizor F) embryos. The number of hours that each gel was run for did not affect the results. The difference in percentage improvement between vigour levels (replicate 3) was larger for the gel run for five hours (Table 3.32) than for the gel run overnight (Table 3.31) although this was not always the case for the other replicates.

Four embryos from each treatment were germinated on filter paper at 24°C in order to determine the health of the embryos following each treatment (Table 3.31). At least three out of the four embryos developed into a seedling for each treatment/replicate. The irradiation treatment may have caused embryo death although one cause of death could have been the non-sterile germination conditions allowing infection by micro-organisms. The control embryos generally produced healthier seedlings than the treated (irradiated and imbibed) embryos. The health of the irradiated-imbibed embryos was, in general, reduced in comparison with the irradiated embryos for the limited number of embryos investigated.

The capacity for DNA repair at 9°C was also investigated using two cv. Cyrano seedlots which had been chosen specifically for their different germination performance at 9°C but similar viability at 20°C (Table 3.33). Embryos from seedlots, cv. Cyrano F (selected for having high vigour) and cv. Cyrano I (selected for low vigour) underwent the same treatments as for the previous experiment except that imbibition took place at a non-optimal temperature of 9°C. Two replicates of all treatments were run on two alkaline gels overnight and another alkaline

gel was run for five hours. The intensity of stained DNA along each lane was quantified using image analysis and the results divided into five segments down each lane (Tables 3.34 and 3.35). The DNA control samples did not show consistent results between the two replicates and when the same samples were run on different gels. The DNA controls could not be run on further gels to verify the results due to limited amounts of the samples. Therefore the repair of only irradiated embryos at 9°C was investigated. In general for both replicates and in the three gels, the higher vigour, cv. Cyrano F embryos showed a greater percentage improvement during imbibition at 9°C following irradiation in comparison with cv. Cyrano I embryos.

The four embryos of each treatment were germinated at 24°C and again the control embryos showed greater development and better health than those embryos which had been irradiated as part of their treatment. Again, imbibition did not improve the health of the irradiated embryos.

TABLE 3.1 The correlation coefficients of the germination performance and the RNA/DNA ratios of the seedlots from cvs. Cyrano, Marathon and Matador (bold indicates a significant ($p<0.05$) correlation).

Germination tests	RNA/DNA ratios		
	1	2	3
9°C-T_{50}	**-0.809**	**-0.800**	**-0.831**
9°C-MGT	**-0.795**	**-0.775**	**-0.762**
9°C-MHT	-0.279	-0.265	-0.317
15°C-T_{50}	**-0.832**	**-0.823**	**-0.784**
15°C-MGT	**-0.654**	**-0.638**	**-0.636**
15°C-MHT	-0.254	-0.260	-0.280
20°C-G% day 3	**0.490**	0.466	0.448
20°C-G% day 6	**0.655**	**0.634**	**0.717**
20°C-G% day 14	**0.624**	**0.603**	**0.697**
20°C-H% day 6	0.306	0.272	0.249
20°C-H% day 14	**0.599**	**0.577**	**0.667**
sand 1-G% day 14	**0.694**	**0.690**	**0.752**
sand 2-G% day 14	**0.637**	**0.633**	**0.698**
wet stress-G% day 4	**0.674**	**0.663**	**0.760**
wet stress-G% day 7	**0.694**	**0.686**	**0.756**
wet stress-G% day 14	**0.690**	**0.686**	**0.759**
wet stress-H% day 4	0.314	0.312	0.426
wet stress-H% day 7	**0.631**	**0.619**	**0.697**
wet stress-H% day 14	**0.652**	**0.650**	**0.722**

TABLE 3.2 The correlation coefficients of the germination performance and the RNA/DNA ratios of the seedlots from cv. Cyrano. No significant correlations were detected.

Germination tests	RNA/DNA ratios		
	1	2	3
9°C-T_{50}	-0.475	-0.408	-0.363
9°C-MGT	-0.549	-0.439	-0.223
9°C-MHT	-0.372	-0.334	-0.527
15°C-T_{50}	-0.467	-0.366	-0.332
15°C-MGT	-0.162	-0.077	-0.227
15°C-MHT	-0.002	-0.008	-0.436
20°C-G% day 3	-0.258	-0.332	-0.541
20°C-G% day 6	-0.049	-0.155	-0.300
20°C-G% day 14	-0.143	-0.243	-0.364
20°C-H% day 6	-0.233	-0.317	-0.444
20°C-H% day 14	-0.129	-0.234	-0.438
sand 1-G% day 14	0.376	0.330	-0.069
sand 2-G% day 14	0.404	0.366	-0.046
wet stress-G% day 4	0.349	0.260	0.280
wet stress-G% day 7	0.385	0.308	0.148
wet stress-G% day 14	0.382	0.308	0.195
wet stress-H% day 4	-0.053	-0.053	0.254
wet stress-H% day 7	0.426	0.332	0.263
wet stress-H% day 14	0.416	0.347	0.287

TABLE 3.3 The correlation coefficients of the germination performance and the RNA/DNA ratios of seedlots from cvs. Marathon and Matador (bold indicates a significant ($p<0.05$) correlation).

Germination tests	RNA/DNA ratios		
	1	2	3
9°C-T_{50}	-0.646	-0.668	-0.588
9°C-MGT	-0.546	-0.558	-0.471
9°C-MHT	0.049	0.060	0.059
15°C-T_{50}	**-0.873**	**-0.883**	**-0.828**
15°C-MGT	-0.631	-0.638	-0.548
15°C-MHT	**-0.865**	**-0.883**	**-0.797**
20°C-G% day 3	0.740	0.746	0.669
20°C-G% day 6	0.224	0.202	0.162
20°C-G% day 14	0.079	0.039	0.063
20°C-H% day 6	0.686	0.680	0.622
20°C-H% day 14	-0.532	-0.568	-0.491
sand 1-G% day 14	-0.363	-0.351	-0.381
sand 2-G% day 14	-0.634	-0.637	-0.635
wet stress-G% day 4	-0.451	-0.436	-0.517
wet stress-G% day 7	-0.279	-0.256	-0.344
wet stress-G% day 14	-0.288	-0.238	-0.353
wet stress-H% day 4	-0.043	-0.048	-0.131
wet stress-H% day 7	-0.465	-0.444	-0.517
wet stress-H% day 14	-0.376	-0.323	-0.441

TABLE 3.4 The correlation coefficients of the germination performance and the RNA/DNA ratios of seedlots from cv. Rizor (bold indicates a significant ($p<0.05$) correlation).

Germination tests	RNA/DNA ratios		
	1	2	3
9°C-T_{50}	-0.403	-0.410	-0.593
9°C-MGT	-0.282	-0.292	-0.453
9°C-MHT	-0.620	-0.593	-0.620
15°C-T_{50}	0.079	0.054	-0.093
15°C-MGT	0.111	0.076	-0.104
15°C-MHT	-0.248	-0.258	-0.417
20°C-G% day 3	**0.653**	**0.704**	**0.722**
20°C-G% day 6	-0.035	0.084	0.160
20°C-G% day 14	0.024	0.148	0.190
20°C-H% day 6	**0.698**	**0.739**	**0.676**
20°C-H% day 14	0.009	0.127	0.115
sand 1-G% day 14	-0.219	-0.169	-0.268
sand 2-G% day 14	-0.304	-0.265	-0.368
wet stress-G% day 4	0.196	0.215	0.294
wet stress-G% day 7	-0.311	-0.231	-0.246
wet stress-G% day 14	-0.493	-0.422	-0.451
wet stress-H% day 4	0.075	0.083	0.176
wet stress-H% day 7	-0.050	0.003	0.049
wet stress-H% day 14	-0.459	-0.388	-0.428

TABLE 3.5 The correlation coefficients of selected germination test measurements of the seedlots from cvs. Cyrano, Marathon and Matador (bold indicates a significant ($p<0.05$) correlation).

	9°C-T_{50}	15°C-T_{50}	15°C-MHT	20°C-G% day 14	20°C-H% day 6	sand 1-G% day 14	wet stress-G% day 14	wet stress-H% day 14
9°C-T_{50}	1							
15°C-T_{50}	**0.783**	1						
15°C-MHT	0.214	**0.525**	1					
20°C-G% day 14	**-0.868**	**-0.569**	0.004	1				
20°C-H% day 6	-0.462	**-0.600**	-0.444	**0.539**	1			
sand 1-G% day 14	**-0.805**	-0.462	0.224	**0.848**	0.165	1		
wet stress-G% day 14	**-0.843**	**-0.526**	0.011	**0.828**	0.279	**0.924**	1	
wet stress-H% day 14	**-0.822**	**-0.488**	0.003	**0.791**	0.240	**0.897**	**0.993**	1

TABLE 3.6 The correlation coefficients of selected germination test measurements of the seedlots from cv. Rizor (bold indicates a significant ($p<0.05$) correlation).

	9°C-T_{50}	15°C-T_{50}	15°C-MHT	20°C-G% day 14	20°C-H% day 6	sand 1-G% day 14	wet stress-G% day 14	wet stress H% day 14
9°C-T_{50}	1							
15°C-T_{50}	**0.696**	**1**						
15°C-MHT	**0.826**	**0.814**	1					
20°C-G% day 14	-0.077	-0.297	-0.121	1				
20°C-H% day 6	-0.102	0.253	0.212	0.425	1			
sand 1-G% day 14	0.468	-0.091	0.192	0.462	0.068	1		
wet stress-G% day 14	0.586	-0.050	0.266	0.595	-0.082	**0.792**	1	
wet stress-H% day 14	0.616	0.009	0.330	0.586	-0.037	**0.735**	**0.982**	1

TABLE 3.7 The percentage increase in the nucleic acids extracted from the embryos following the advancement treatment in comparison with those extracted from steeped seeds.

Lot	DNA	RNA (numbers correspond to RNA/DNA calculation)			
		1	2	3	4
B	35	100	105	135	105
G	27	79	81	87	81
H	20	47	49	57	50

TABLE 3.8 The correlation coefficients of the germination performance of the advanced or thiram-steeped cv. Cyrano lots at 9°C and 20°C correlated with the RNA/DNA ratios (bold indicates a significant ($p<0.05$) correlation).

Germination tests	RNA/DNA ratios			
	1	2	3	4
9°C-MGT	**-0.933**	**-0.933**	**-0.899**	**-0.928**
9°C-MHT	**-0.952**	**-0.952**	**-0.916**	**-0.947**
20°C-G% day 3	0.360	0.368	0.437	0.376
20°C-G% day 6	0.114	0.118	0.103	0.113
20°C-G% day 14	0.014	0.017	-0.001	0.011

TABLE 3.9 The correlation coefficients of the germination performance and the RNA/DNA ratios of selected seedlots from cvs. Cyrano, Rizor and Matador (seedlots 2 experiment). No significant correlations were detected.

Germination tests	RNA/DNA ratios						
	1	2	3	4	5+6[1]	7 + 8[1]	9 + 10[1]
9°C-T_{50}	-0.352	-0.335	-0.060	-0.279	-0.399	-0.362	-0.361
9°C-T_{30}	-0.331	-0.323	-0.192	-0.265	-0.358	-0.399	-0.322
9°C-T_{50}*	-0.369	-0.362	-0.182	-0.313	-0.413	-0.453	-0.386
9°C-MGT	-0.319	-0.318	-0.239	-0.273	-0.365	-0.467	-0.345
9°C-MHT	-0.543	-0.548	-0.098	-0.569	-0.624	-0.588	-0.652
20°C-G% day 4	0.276	0.248	-0.174	0.208	0.217	-0.070	0.169
20°C-G% day 7	0.278	0.246	-0.224	0.203	0.230	-0.082	0.177
20°C-G% day 15	0.284	0.253	-0.226	0.210	0.237	-0.079	0.184
20°C-H% day 4	0.267	0.287	0.482	0.255	0.317	0.622	0.320
20°C-H% day 7	0.290	0.253	-0.300	0.208	0.257	-0.095	0.202
20°C-H% day 15	0.342	0.306	-0.298	0.266	0.308	-0.064	0.255
sand 1-G% day 14	0.542	0.495	-0.389	0.448	0.570	0.132	0.514
wet stress-G% day 2	0.646	0.641	0.219	0.607	0.643	0.548	0.622
wet stress-G% day 4	0.375	0.344	-0.198	0.298	0.342	0.031	0.290
wet stress-G% day 7	0.382	0.353	-0.160	0.310	0.338	0.037	0.288
wet stress-G% day 14	0.416	0.384	-0.188	0.339	0.379	0.056	0.328
wet stress-H% day 4	0.468	0.482	0.406	0.460	0.516	0.684	0.521
wet stress-H% day 7	0.467	0.438	-0.118	0.388	0.436	0.151	0.384
wet stress-H% day 14	0.455	0.420	-0.237	0.373	0.432	0.076	0.379

[1] both RNA/DNA ratio calculations generate the same correlation coefficients

TABLE 3.10 The correlation coefficients of selected germination test measurements of selected seedlots (seedlots 2 experiment) from cvs. Cyrano, Rizor and Matador (bold indicates a significant ($p<0.05$) correlation).

	9°C-T_{50}	20°C-G% day15	20°C-H% day15	sand 1-G% day14	wet stress-G% day14	wet stress-H% day4	wet stress-H% day 14
9°C-T_{50}	1						
20°C-G% day 15	-0.059	1					
20°C-H% day 15	-0.056	**0.988**	1				
sand 1-G% day 14	-0.450	0.756	**0.825**	1			
wet stress-G% day 14	-0.181	**0.984**	**0.987**	**0.841**	1		
wet stress-H% day 4	**-0.845**	-0.339	-0.330	0.114	-0.191	1	
wet stress-H% day 14	-0.243	**0.961**	**0.979**	**0.900**	**0.993**	-0.131	1

TABLE 3.11 The correlation coefficients of the field performance (trial 1) and the RNA/DNA ratios of the seedlots from cvs. Cyrano, Marathon and Matador (bold indicates a ($p<0.05$) significant correlation).

Field trial measurements	RNA/DNA ratios		
	1	2	3
sum%	**0.520**	**0.527**	**0.649**
establishment%	**0.523**	**0.527**	**0.651**
MET	**-0.528**	**-0.554**	**-0.583**
T_{50}	-0.472	-0.479	**-0.590**
T_{50}*	-0.471	**-0.490**	**-0.513**
T_{30}	**-0.533**	**-0.546**	**-0.615**

TABLE 3.12 The correlation coefficients of the field performance and the RNA/DNA ratios of the seedlots from cv. Cyrano. No significant correlations were detected.

Field trial measurements	RNA/DNA ratios		
	1	2	3
sum%	-0.473	-0.457	-0.275
establishment%	-0.496	-0.491	-0.324
MET	0.156	0.086	-0.139
T_{50}	0.314	0.293	0.166
T_{50}*	0.344	0.292	0.093
T_{30}	0.329	0.300	0.122

TABLE 3.13 The correlation coefficients of the field performance and the RNA/DNA ratios of the seedlots from cvs. Marathon and Matador. No significant correlations were detected.

Field trial measurements	RNA/DNA ratios		
	1	2	3
sum%	-0.070	-0.044	-0.152
establishment%	-0.031	-0.004	-0.104
MET	-0.650	-0.679	-0.583
T_{50}	-0.292	-0.320	-0.199
T_{50}*	-0.580	-0.609	-0.519
T_{30}	-0.538	-0.566	-0.470

TABLE 3.14 The correlation coefficients of the laboratory performance and field assessment of the seedlots from cvs. Cyrano, Marathon and Matador (bold indicates a significant ($p<0.05$) correlation).

Germination tests	Field trial measurements					
	sum%	establishment%	MET	T_{50}	T_{50}*	T_{30}
9°C-T_{50}	**-0.787**	**-0.783**	**0.589**	**0.624**	**0.517**	**0.690**
9°C-MGT	**-0.763**	**-0.758**	**0.620**	**0.643**	**0.564**	**0.723**
9°C-MHT	-0.360	-0.366	0.129	0.231	-0.005	0.217
15°C-T_{50}	**-0.579**	**-0.584**	**0.709**	**0.542**	**0.707**	**0.700**
15°C-MGT	**-0.578**	**-0.560**	**0.651**	**0.551**	**0.614**	**0.671**
15°C-MHT	-0.145	-0.143	**0.574**	0.280	**0.532**	0.449
20°C-G% day 3	**0.658**	**0.658**	**-0.649**	**-0.720**	**-0.644**	**-0.752**
20°C-G% day 6	**0.812**	**0.811**	-0.459	**-0.681**	-0.414	**-0.650**
20°C-G% day 14	**0.803**	**0.804**	-0.439	**-0.688**	-0.386	**-0.634**
20°C-H% day 6	0.482	**0.488**	**-0.539**	**-0.644**	**-0.525**	**-0.615**
20°C-H% day 14	**0.789**	**0.792**	-0.387	**-0.656**	-0.339	**-6.01**
sand 1-G% day 14	**-0.781**	**0.782**	-0.314	**-0.557**	-0.258	**-0.489**
sand 2-G% day 14	**0.741**	**0.739**	-0.239	**-0.522**	-0.182	-0.422
wet stress-G% day 4	**0.839**	**0.842**	-0.435	**-0.704**	-0.361	**-0.598**
wet stress-G% day 7	**0.853**	**0.859**	-0.440	**-0.695**	-0.363	**-0.605**
wet stress-G% day 14	**0.844**	**0.851**	-0.465	**-0.718**	-0.396	**-0.620**
wet stress-H% day 4	**0.650**	**0.627**	**-0.588**	**-0.583**	**-0.484**	**-0.655**
wet stress-H% day 7	**0.787**	**0.794**	-0.336	**-0.642**	-0.246	**-0.511**
wet stress-H% day 14	**0.813**	**0.819**	-0.432	**-0.692**	-0.358	**-0.576**

TABLE 3.15 The correlation coefficients of the laboratory performance and field assessment of the seedlots from cv. Cyrano (bold indicates a significant ($p<0.05$) correlation).

Germination tests	Field trial measurements					
	sum%	establishment%	MET	T_{50}	T_{50}*	T_{30}
9°C-T_{50}	-0.120	-0.097	0.040	-0.070	-0.182	0.044
9°C-MGT	-0.077	-0.088	0.084	0.054	-0.083	0.0140
9°C-MHT	-0.219	-0.201	0.222	0.080	0.047	0.270
15°C-T_{50}	0.098	0.083	-0.014	0.094	-0.114	-0.016
15°C-MGT	-0.138	-0.148	0.170	0.240	0.038	0.207
15°C-MHT	-0.354	-0.340	0.583	0.447	0.552	0.547
20°C-G% day 3	0.487	0.502	-0.330	-0.576	-0.372	-0.525
20°C-G% day 6	0.321	0.322	-0.093	-0.113	-0.045	-0.294
20°C-G% day 14	0.335	0.340	-0.087	-0.168	-0.011	-0.287
20°C-H% day 6	0.500	0.528	-0.370	**-0.678**	-0.396	-0.550
20°C-H% day 14	0.306	0.312	-0.088	-0.106	-0.073	-0.317
sand 1-G% day 14	-0.091	-0.094	0.340	0.522	0.388	0.305
sand 2-G% day 14	-0.069	-0.072	0.295	0.480	0.329	0.275
wet stress-G% day 4	0.253	0.287	-0.171	-0.175	-0.061	-0.181
wet stress-G% day 7	0.256	0.295	-0.091	-0.112	0.053	-0.124
wet stress-G% day 14	0.355	0.390	-0.147	-0.241	-0.013	-0.193
wet stress-H% day 4	**0.673**	**0.652**	**-0.791**	-0.434	**-0.725**	**-0.798**
wet stress-H% day 7	0.162	0.206	-0.021	-0.093	0.114	-0.081
wet stress-H% day 14	0.282	0.315	-0.098	-0.218	0.061	-0.112

TABLE 3.16 The correlation coefficients of the laboratory performance and field assessment of the seedlots from cvs. Marathon and Matador (bold indicates a significant ($p<0.05$) correlation).

Germination tests	Field trial measurements					
	sum%	establishment%	MET	T_{50}	T_{50}*	T_{30}
9°C-T_{50}	-0.652	-0.666	**0.900**	**0.840**	**0.838**	**0.929**
9°C-MGT	-0.711	-0.682	**0.846**	**0.907**	**0.792**	**0.900**
9°C-MHT	-0.413	-0.472	-0.215	0.156	-0.286	-0.160
15°C-T_{50}	-0.382	-0.405	**0.878**	0.707	**0.823**	**0.833**
15°C-MGT	-0.458	-0.398	**0.764**	0.746	0.702	**0.764**
15°C-MHT	-0.310	-0.323	**0.796**	0.626	0.714	0.711
20°C-G% day 3	0.498	0.472	**-0.855**	**-0.803**	**-0.798**	**-0.850**
20°C-G% day 6	0.297	0.202	-0.279	-0.473	-0.260	-0.316
20°C-G% day 14	0.048	-0.053	-0.008	-0.151	0.000	-0.076
20°C-H% day 6	0.455	0.429	-0.698	-0.728	-0.642	-0.693
20°C-H% day 14	-0.200	-0.223	**0.836**	0.553	**0.874**	0.750
sand 1-G% day 14	0.151	0.169	0.480	0.230	0.581	0.433
sand 2-G% day 14	-0.116	-0.138	**0.776**	0.496	**0.826**	0.731
wet stress-G% day 4	0.566	0.527	0.220	-0.222	0.302	0.104
wet stress-G% day 7	0.730	0.739	0.031	-0.388	0.127	-0.081
wet stress-G% day14	0.417	0.423	-0.062	-0.198	-0.005	-0.091
wet stress-H% day 4	0.343	0.232	-0.074	-0.369	-0.024	-0.119
wet stress-H% day 7	0.380	0.374	0.367	-0.005	0.453	0.298
wet stress-H% day14	0.354	0.357	0.001	-0.140	0.038	-0.013

TABLE 3.17 The correlation coefficients of field performance (trial 2) correlated with the RNA/DNA ratios of steeped and advanced seedlots from cv. Cyrano (bold indicates a significant ($p<0.01$) correlation).

Field trial measurements	RNA/DNA ratios			
	1	2	3	4
sum%	**0.984**	**0.982**	**0.970**	**0.983**
establishment%	**0.987**	**0.985**	**0.968**	**0.985**
MET	**-0.971**	**-0.972**	**-0.959**	**-0.970**
T_{50}	**-0.981**	**-0.982**	**-0.988**	**-0.983**

TABLE 3.18 The correlation coefficients of the performance of steeped and advanced seedlots from cv. Cyrano in the germination tests at 9°C and 20°C and in field trial 2 (bold indicates a significant correlation ($p<0.05$).

Germination tests	Field trial measurements			
	sum%	establishment%	MET	T_{50}
9°C-MGT	**-0.871**	**-0.884**	**0.955**	**0.883**
9°C-MHT	**-0.900**	**-0.913**	**0.975**	**0.911**
20°C-G% day 3	0.417	0.385	-0.188	-0.365
20°C-G% day 6	0.132	0.113	-0.079	-0.097
20°C-G% day 14	0.038	0.018	0.010	-0.007

TABLE 3.19 The correlation coefficients of the RNA/DNA ratios and the field performance of seedlots from cv. Rizor. No significant correlations were detected.

Field trial measurements	RNA/DNA ratios		
	1	2	3
establishment%	-0.205	-0.121	0.053
MET	-0.423	-0.453	-0.418
T_{50}	-0.125	-0.190	-0.161
T_{50}*	-0.486	-0.480	-0.427
T_{30}	-0.357	-0.368	-0.386

TABLE 3.20 The correlation coefficients of the performance of seedlots from cv. Rizor in germination tests and field trial 3 (bold indicates a significant ($p<0.05$) correlation).

Germination tests	Field trial measurements				
	establishment%	MET	T_{50}	T_{50}*	T_{30}
9°C-T_{50}	-0.151	0.341	-0.007	0.459	0.338
9°C-MGT	-0.086	0.130	-0.092	0.385	0.041
9°C-MHT	0.199	0.119	-0.177	0.440	0.038
15°C-T_{50}	-0.236	-0.193	-0.273	-0.082	-0.112
15°C-MGT	-0.294	-0.075	-0.179	0.023	-0.032
15°C-MHT	-0.051	-0.107	-0.356	0.154	-0.113
20°C-G% day 3	0.448	-0.305	-0.466	0.001	-0.261
20°C-G% day 6	0.616	-0.040	-0.339	0.201	0.013
20°C-G% day 14	0.609	-0.250	-0.536	0.036	-0.154
20°C-H% day 6	0.327	**-0.633**	**-0.694**	-0.284	-0.573
20°C-H% day 14	0.536	-0.208	-0.556	0.053	-0.073
sand 1-G% day 14	0.318	0.477	0.004	**0.641**	0.622
sand 2-G% day 14	0.294	0.518	0.050	**0.651**	**0.724**
wet stress-G% day 4	0.466	0.004	-0.312	0.375	-0.163
wet stress-G% day 7	0.482	0.331	-0.206	0.584	0.359
wet stress-G% day 14	0.342	0.470	-0.032	**0.668**	0.406
wet stress-H% day 4	0.143	0.155	0.194	-0.030	0.122
wet stress-H% day 7	0.507	0.196	-0.262	0.550	0.016
wet stress-H% day 14	0.340	0.421	-0.048	**0.672**	0.328

TABLE 3.21 The correlation coefficients of the RNA/DNA ratios and the field performance of selected seedlots from cvs. Cyrano, Rizor and Matador (seedlots 2 experiment). No significant correlations were detected.

RNA/DNA ratios	Field trial measurements				
	sum %	establishment %	MET	T_{30}	T_{50}*
1	0.218	0.130	-0.480	-0.468	-0.238
2	0.156	0.066	-0.495	-0.429	-0.226
3	-0.723	-0.797	-0.314	0.193	0.004
4	0.120	0.036	-0.492	-0.361	-0.194
5+6[1]	0.203	0.116	-0.559	-0.491	-0.327
7+8[1]	-0.406	-0.513	-0.697	-0.209	-0.337
9+10[1]	0.137	0.052	-0.579	-0.429	-0.311

[1] both RNA/DNA ratio calculations generate the same correlation coefficients

TABLE 3.22 The correlation coefficients of the RNA/DNA ratios and the fresh and dry seedling weights of selected seedlots (seedlots 2 experiment) from cvs. Cyrano, Rizor and Matador (bold indicates a significant ($p<0.05$) correlation). The weights of harvested seedlings which emerged: early (a), in the middle (b) and late (c) in the counting period.

Seedling weights	RNA/DNA ratios						
	1	2	3	4	5+6[1]	7+8[1]	9+10[1]
fresh a	0.175	0.168	0.066	0.133	0.260	0.340	0.247
fresh b	-0.274	-0.300	-0.540	-0.277	-0.317	-0.646	-0.328
fresh c	0.113	0.057	-0.570	0.001	0.123	-0.306	0.053
total fresh	-0.249	-0.304	**-0.858**	-0.307	-0.245	-0.754	-0.286
fresh/plant a	-0.682	-0.661	-0.074	-0.606	-0.655	-0.423	-0.607
fresh/plant b	-0.529	-0.533	-0.341	-0.493	-0.570	-0.663	-0.556
fresh/plant c	0.386	0.369	0.038	0.317	0.449	0.411	0.416
total fresh/plant	-0.602	-0.609	-0.426	-0.566	-0.574	-0.615	-0.552
dry a	0.198	0.190	0.059	0.152	0.281	0.344	0.264
dry b	-0.218	-0.249	-0.579	-0.233	-0.261	-0.640	-0.280
dry c	0.165	0.110	-0.541	0.051	0.167	-0.272	0.094
total dry	-0.097	-0.163	**-0.906**	-0.186	-0.086	-0.677	-0.145
dry/plant a	-0.733	-0.710	-0.050	-0.656	-0.719	-0.466	-0.672
dry/plant b	-0.542	-0.555	-0.430	-0.525	-0.586	-0.740	-0.585
dry/plant c	0.414	0.394	0.016	0.338	0.471	0.398	0.433
total dry/plant	-0.531	-0.563	-0.657	-0.551	-0.460	-0.639	-0.468

[1] both RNA/DNA ratio calculations generate the same correlation coefficients

TABLE 3.23 The correlation coefficients of the seedling weights and the field emergence of selected seedlots (seedlots 2 experiment) from cvs. Cyrano, Rizor and Matador (bold indicates a significant ($p<0.05$) correlation). The weights of harvested seedlings which emerged: early (a), in the middle (b) and late (c) in the counting period.

Seedling weights	Field trial measurements				
	sum%	establishment%	MET	T_{30}	T_{50}*
fresh a	-0.290	-0.350	**-0.846**	-0.584	**-0.992**
fresh b	0.572	0.657	**0.841**	0.431	**0.831**
fresh c	**0.861**	0.794	0.242	-0.707	-0.034
total fresh	0.791	**0.857**	0.644	0.054	0.463
fresh/plant a	-0.457	-0.338	0.207	**0.882**	0.314
fresh/plant b	0.198	0.312	**0.814**	0.739	**0.850**
fresh/plant c	0.104	-0.020	-0.672	**-0.848**	-0.792
total fresh/plant	0.023	0.131	0.420	0.701	0.468
dry a	-0.256	-0.320	**-0.834**	-0.622	**-0.991**
dry b	0.652	0.728	**0.838**	0.338	0.804
dry c	**0.875**	0.804	0.249	-0.747	-0.033
total dry	**0.912**	**0.948**	0.516	-0.246	0.260
dry/plant a	-0.453	-0.330	0.299	**0.904**	0.385
dry/plant b	0.325	0.433	**0.881**	0.625	**0.847**
dry/plant c	0.160	0.034	-0.637	**-0.891**	-0.777
total dry/plant	0.228	0.297	0.185	0.254	0.075

TABLE 3.24 The correlation coefficients of the germination test results and field performance of selected seedlots (seedlots 2 experiment) from cvs. Cyrano, Rizor and Matador (bold indicates a significant ($p<0.05$) correlation).

Germination tests	Field trial measurements				
	sum%	establishment %	MET	T_{30}	T_{50}*
9°C-T_{50}	-0.002	0.092	0.659	**0.856**	**0.877**
9°C-T_{30}	0.043	0.147	0.581	**0.838**	0.790
9°C-T_{50}*	0.139	0.230	0.721	0.777	**0.890**
9°C-MGT	0.242	0.328	0.754	0.707	**0.905**
9°C-MHT	0.335	0.349	**0.888**	0.100	0.663
20°C-G% day 4	0.780	0.696	0.479	-0.492	0.394
20°C-G% day 7	0.805	0.717	0.431	-0.549	0.323
20°C-G% day 15	0.808	0.721	0.431	-0.542	0.329
20°C-H% day 4	-0.382	-0.506	-0.778	-0.550	-0.759
20°C-H% day 7	**0.837**	0.749	0.367	-0.585	0.252
20°C-H% day 15	**0.846**	0.762	0.366	-0.552	0.286
sand 1-G% day 14	0.746	0.646	-0.159	-0.803	-0.257
wet stress-G% day 2	-0.025	-0.091	-0.544	-0.649	-0.610
wet stress-G% day 4	0.779	0.675	0.271	-0.638	0.185
wet stress-G% day 7	0.773	0.672	0.328	-0.593	0.259
wet stress-G% day 14	0.784	0.680	0.267	-0.640	0.192
wet stress-H% day 4	-0.431	-0.510	**-0.899**	-0.514	**-0.880**
wet stress-H% day 7	0.722	0.603	0.147	-0.726	0.068
wet stress-H% day 14	0.797	0.691	0.179	-0.692	0.099

TABLE 3.25 The correlation coefficients of the germination test results and the fresh seedling weights of selected seedlots (seedlots 2 experiment) from cvs. Cyrano, Rizor and Matador (bold indicates a significant ($p<0.05$) correlation). The weights of harvested seedlings which emerged: early (a), in the middle (b) and late (c) in the counting period.

Germination tests	fresh a	fresh b	fresh c	total fresh	fresh/plant a	fresh/plant b	fresh/plant c	total fresh/plant
9°C-T_{50}	**-0.886**	0.806	-0.294	0.456	0.691	**0.935**	**-0.877**	0.769
9°C-T_{30}	**-0.816**	**0.820**	-0.261	0.537	0.736	**0.930**	**-0.834**	**0.848**
9°C-T_{50}*	**-0.901**	**0.880**	-0.142	0.584	0.648	**0.962**	**-0.832**	0.802
9°C-MGT	**-0.922**	**0.921**	-0.051	0.646	0.571	**0.958**	-0.806	0.773
9°C-MHT	-0.600	0.526	0.331	0.382	0.004	0.519	-0.381	0.143
20°C-G% day 4	-0.381	0.387	0.803	0.437	-0.619	0.062	0.189	-0.223
20°C-G% day 7	-0.311	0.357	**0.854**	0.455	-0.635	0.018	0.261	-0.222
20°C-G% day 15	-0.319	0.365	**0.851**	0.461	-0.630	0.025	0.255	-0.214
20°C-H% day 4	**0.817**	**-0.896**	0.062	-0.688	-0.409	**-0.886**	**0.864**	-0.574
20°C-H% day 7	-0.246	0.348	**0.903**	0.502	-0.620	-0.007	0.328	-0.178
20°C-H% day 15	-0.288	0.383	**0.877**	0.516	-0.610	0.020	0.290	-0.161
sand 1-G% day 14	0.228	0.006	**0.871**	0.352	-0.694	-0.382	0.685	-0.292
wet stress-G% day 2	0.577	-0.669	-0.022	-0.552	-0.757	-0.802	0.493	**-0.916**
wet stress-G% day 4	-0.176	0.238	**0.874**	0.387	-0.692	-0.118	0.406	-0.282

wet stress-G% day 7	-0.250	0.276	**0.835**	0.379	-0.692	-0.074	0.332	-0.293
wet stress-G% day 14	-0.187	0.238	**0.858**	0.375	-0.716	-0.125	0.392	-0.310
wet stress-H% day 4	**0.882**	**-0.959**	-0.209	-0.784	-0.454	**-0.945**	0.699	-0.730
wet stress-H% day 7	-0.058	0.093	**0.844**	0.258	-0.789	-0.267	0.506	-0.422
wet stress-H% day 14	-0.102	0.195	**0.884**	0.382	-0.728	-0.184	0.467	-0.311

TABLE 3.26 The correlation coefficients of the germination test results and the dry seedling weights of selected seedlots (seedlots 2 experiment) from cvs. Cyrano, Rizor and Matador (bold indicates a significant ($p<0.05$) correlation). The weights of harvested seedlings which emerged: early (a), in the middle (b) and late (c) in the counting period.

Germination tests	dry a	dry b	dry c	total dry	dry/plant a	dry/plant b	dry/plant c	total dry/plant
9°C-T_{50}	**-0.909**	0.746	-0.331	0.180	0.721	**0.875**	**-0.897**	0.354
9°C-T_{30}	**-0.843**	0.761	-0.310	0.267	0.745	**0.867**	**-0.858**	0.484
9°C-T_{50}*	**-0.921**	**0.830**	-0.184	0.326	0.677	**0.923**	**-0.847**	0.440
9°C-MGT	**-0.938**	**0.881**	-0.089	0.406	0.601	**0.933**	**-0.813**	0.433
9°C-MHT	-0.581	0.532	0.348	0.325	0.112	0.611	-0.344	0.014
20°C-G% day 4	-0.342	0.459	**0.831**	0.547	-0.584	0.178	0.240	-0.110
20°C-G% day 7	-0.272	0.433	**0.877**	0.582	-0.608	0.139	0.310	-0.060

20°C-G% day 15	-0.280	0.441	**0.874**	0.586	-0.604	0.144	0.304	-0.056
20°C-H% day 4	**0.818**	**-0.879**	0.058	-0.518	-0.458	**-0.894**	**0.837**	-0.263
20°C-H% day 7	-0.210	0.427	**0.916**	0.639	-0.608	0.114	0.372	0.035
20°C-H% day 15	-0.252	0.460	**0.891**	0.643	-0.602	0.134	0.334	0.027
sand 1-G% day 14	0.257	0.094	**0.871**	0.568	-0.747	-0.284	0.712	0.106
wet stress-G% day 2	0.609	-0.612	0.064	-0.320	-0.760	-0.776	0.540	-0.757
wet stress-G% day 4	-0.138	0.319	**0.893**	0.542	-0.684	-0.003	0.449	-0.060
wet stress-G% day 7	-0.211	0.355	**0.861**	0.521	-0.674	0.039	0.379	-0.118
wet stress-G% day 14	-0.148	0.320	**0.882**	0.533	-0.707	-0.011	0.438	-0.100
wet stress-H% day 4	**0.887**	**-0.938**	-0.174	-0.595	-0.502	**-0.973**	0.692	-0.498
wet stress-H% day 7	-0.017	0.178	**0.873**	0.444	-0.785	-0.154	0.551	-0.173
wet stress-H% day 14	-0.064	0.280	**0.903**	0.556	-0.733	-0.071	0.510	-0.054

TABLE 3.27 The correlation coefficients of the RNA/DNA ratios correlated with seed size measurements and field trial data of size-graded fruits (data provided by Thomas and Yallop, IACR-Broom's Barn; bold indicates a significant ($p<0.05$) correlation; TSW=mean dried true seed weight (mg), 1000WT = the weight of 1000 fruits (g), DW/PL 1=dry weight per plant (g) of first harvest and DW/PL 2 of second harvest, TTFRWT=total fresh weight (g), TOPDRW=% of fresh weight of leaves that is dry matter, ROTDRW= root dry weight (g)).

Seed size measurements	RNA/DNA ratios			
	1	2	3	4
TSW	**0.845**	**0.837**	0.695	**0.827**
1000WT	**0.829**	**0.820**	0.669	**0.814**
MET	**-0.822**	**-0.836**	-0.747	**-0.833**
T_{50}	**-0.867**	**-0.868**	-0.762	**-0.864**
establishment%	0.220	0.223	0.234	0.215
DW/PL 1	**0.904**	**0.913**	**0.816**	**0.898**
DW/PL 2	**0.989**	**0.982**	**0.930**	**0.973**
TTFRWT	0.424	0.420	0.278	0.366
TOPDRW	**0.947**	**0.951**	**0.930**	**0.934**
ROTDRW	0.447	0.416	0.305	0.375

TABLE 3.28 The percentage of cells with a DNA content of 4C or larger than 4C.

Tissue	DNA content at 2C peak	4C DNA content (2C peak x 2)	% cells with DNA content of 4C	% cells with DNA contents >4C
onion, root tip	107,500	215,000	2	1
Cyrano G, leaf	7250	14,500	2	1
Cyrano G, root tip, untreated	6750	13,500	2	1
Cyrano G, root tip, aged	5250	use 4C DNA content of untreated Cyrano G root tip cells ie. 13,500	2	0
Cyrano G, root tip, advanced	5250		14	10
Cyrano G, root tip, advanced/aged	5000		5	11
Cyrano B, root tip, untreated	4750	9500	5	0
Cyrano H, root tip, untreated	3000	6000	11	9

TABLE 3.29 The estimation of DNA per nucleus in 2C cells using *Allium cepa* as the standard.

Tissue	2C peak (range)	x $\frac{33.5}{107,500}$ = amount of DNA (pg) per nucleus in 2C cell
Cyrano G, leaf	7250 (7000-7500)	2.259
Cyrano G, root tip	6750 (6500-7000)	2.103
Cyrano H, root tip	3000 (2500-3500)	0.935
Cyrano B, root tip	4750 (4500-5000)	1.48

TABLE 3.30 The capacity of cv. Cyrano C embryos to repair DNA (fragmented by γ-irradiation) during a two hour imbibition at 24°C. The gel was divided into four segments down the gel. The percentage of the total intensity of ethidium bromide-stained DNA in each segment of an alkaline gel run overnight is shown. Segment A was found at the top of the gel nearest the origin; subsequent segments were positioned down the gel.

segment	control	irradiated	irradiated-imbibed
A	19.23	10.93	19.12
B	30.77	27.65	26.89
C	30.77	34.09	28.78
D	19.23	27.33	25.21
total	100	100	100

TABLE 3.31 The capacity of high vigour (cv. Rizor B) and low vigour (cv. Rizor F) embryos to repair DNA during a two hour imbibition at 24°C. The gel was divided into five segments down the gel. The percentage of the total intensity of ethidium bromide-stained DNA in each segment is shown for three replicates of samples run overnight on alkaline gels. Segment A was found at the top of the gel nearest the origin; subsequent segments were positioned down the gel. The number of embryos (four per treatment) that developed into a seedling are shown along with the total of the health rankings (0=four dead embryos, 16=four healthy seedlings with greening cotyledons and a developing root) for each group of four embryos.

REPLICATE 1	Rizor B			Rizor F		
segment	control	irradiated	irradiated-imbibed	control	irradiated	irradiated-imbibed
A	8.16	8.23	11.03	12.20	9.49	18.35
B	18.68	18.67	18.69	22.67	20.34	23.48
C	25.03	23.99	23.32	23.03	24.02	21.63
D	24.66	25.55	24.59	22.41	23.27	19.71
E	23.47	23.56	22.37	19.69	22.89	16.83
total	100	100	100	100	100	100
% improvement			34.03			93.36
germination at 24°C (out of 4)	4	4	3	4	3	3
total health ranking	15	9	4	14	8	5

REPLICATE 3	Rizor B			Rizor F		
segment	control	irradiated	irradiated-imbibed	control	irradiated	irradiated-imbibed
A	17.07	8.42	12.93	23.74	16.33	20.49
B	21.42	20.01	21.91	23.65	22.98	24.08
C	21.90	23.93	23.48	20.70	22.79	22.31
D	21.64	24.22	22.28	18.77	21.13	18.85
E	17.98	23.42	19.39	13.14	16.78	14.27
total	100	100	100	100	100	100
% improvement			53.56			25.47
germination at 24°C (out of 4)	4	3	3	4	4	3
total health ranking	13	5	4	16	14	4

REPLICATE 4	Rizor B			Rizor F		
segment	control	irradiated	irradiated-imbibed	control	irradiated	irradiated-imbibed
A	20.20	13.10	20.12	18.03	6.90	11.83
B	24.36	24.98	24.94	24.06	19.64	22.49
C	21.82	24.36	22.45	21.97	24.40	23.64
D	18.18	20.53	18.05	19.40	24.72	22.51
E	15.44	17.03	14.43	16.54	24.35	19.54
total	100	100	100	100	100	100
% improvement			53.59			71.45
germination at 24°C (out of 4)	4	4	4	4	3	4
total health ranking	12	12	7	11	6	8

TABLE 3.32 The capacity of high vigour (cv. Rizor B) and low vigour (cv. Rizor F) embryos to repair irradiated DNA during a two hour imbibition at 24°C. The gel was divided into five segments down the gel. The percentage of the total intensity of ethidium bromide-stained DNA in each segment is shown for two replicates of samples run for five hours on alkaline gels. Segment A was found at the top of the gel nearest the origin; subsequent segments were positioned down the gel. The number of embryos (four per treatment) that developed into a seedling are shown along with the total of the health rankings explained in Table 3.31.

REPLICATE 2	Rizor B			Rizor F		
segment	control	irradiated	irradiated-imbibed	control	irradiated	irradiated-imbibed
A	24.48	11.35	9.68	24.19	6.95	7.89
B	22.42	20.51	19.87	22.14	17.95	17.40
C	20.97	22.37	22.43	20.34	22.81	24.29
D	18.04	24.24	24.73	18.69	25.97	25.68
E	14.09	21.54	23.29	14.64	26.32	24.75
total	100	100	100	100	100	100
% improvement			none			13.52

REPLICATE 3	Rizor B			Rizor F		
segment	control	irradiated	irradiated-imbibed	control	irradiated	irradiated-imbibed
A	19.51	8.81	14.73	21.73	16.40	19.18
B	23.68	16.03	22.15	23.92	22.59	22.91
C	23.13	24.59	23.88	22.86	24.92	24.22
D	18.82	25.53	20.62	17.41	18.81	18.03
E	14.87	25.03	18.62	14.08	17.28	15.67
total	100	100	100	100	100	100
% improvement			67.20			16.95

TABLE 3.33 The germination percentages of a cv. Cyrano seedlot selected for high vigour (cv. Cyrano F) and a lot selected for low vigour (cv. Cyrano I). These selections were based on their different germination percentages tested in standard conditions at 9°C and their similar viability tested in standard germination tests at 20°C using rubbed and thiram-steeped seeds (also Appendix C1). The seeds used in the DNA repair treatment were raw so that the germination tests took place using untreated seeds.

Cyrano lot	rubbed and thiram-steeped				untreated			
	9°C		20°C		9°C		24°C	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
I	64.67	2.85	77.67	5.55	59.33	5.61	77.33	2.33
F	83.33	2.33	82.00	0.58	71.33	2.91	81.00	0.58

TABLE 3.34 The capacity of high vigour (cv. Cyrano F) and low vigour (cv. Cyrano I) embryos to repair irradiated DNA during a two hour imbibition at 9°C. Each gel was divided into five segments. The percentage of the total intensity of ethidium bromide-stained DNA in each segment is shown for two replicates of samples run overnight on two alkaline gels. Segment A was found at the top of the gel nearest the origin; subsequent segments were positioned down the gel. The number of embryos (four per treatment) that developed into a seedling are shown along with the total of the health rankings (0=four dead embryos; 16=four healthy seedlings with greening cotyledons and a developing root) for each group of four embryos.

GEL 1	replicate 1				replicate 2			
	Cyrano F		Cyrano I		Cyrano F		Cyrano I	
segment	irradiated	irradiated-imbibed	irradiated	irradiated-imbibed	irradiated	irradiated-imbibed	irradiated	irradiated-imbibed
A	8.69	20.65	14.31	12.08	13.33	14.89	18.76	24.04
B	17.19	25.97	22.78	20.95	21.35	24.25	24.81	26.88
C	24.79	21.51	23.65	24.25	22.80	23.84	21.90	22.17
D	26.48	17.65	20.99	22.35	21.97	20.61	18.66	16.02
E	22.86	14.22	18.27	20.36	20.55	16.41	15.87	10.89
total	100	100	100	100	100	100	100	100
% improvement		137.63		none		11.70		28.15
germination at 24°C (out of 4)	4	2	2	2				
total health ranking	5	4	3	2				

GEL 2	replicate 1				replicate 2			
	Cyrano F		Cyrano I		Cyrano F		Cyrano I	
segment	irradiated	irradiated-imbibed	irradiated	irradiated-imbibed	irradiated	irradiated-imbibed	irradiated	irradiated-imbibed
A	14.70	26.08	16.37	14.05	10.25	26.81	20.88	22.79
B	24.92	26.22	26.21	23.25	24.49	28.84	27.18	27.62
C	25.70	22.66	27.08	28.14	26.90	22.63	26.72	24.09
D	20.77	15.29	19.28	21.86	24.29	14.93	17.38	16.38
E	13.92	9.76	11.06	12.70	14.06	6.79	7.84	9.12
total	100	100	100	100	100	100	100	100
% improvement		77.41		none		161.56		9.15

TABLE 3.35 The capacity of high vigour (cv. Cyrano F) and low vigour (cv. Cyrano I) embryos to repair irradiated DNA during a two hour imbibition at 9°C. The gel was divided into five segments. The percentage of the total intensity of ethidium bromide-stained DNA in each segment is shown for two replicates of samples run for five hours on an alkaline gel. Segment A was found at the top of the gel nearest the origin; subsequent segments were positioned down the gel.

GEL 1	replicate 1				replicate 2			
	Cyrano F		Cyrano I		Cyrano F		Cyrano I	
segment	irradiated	irradiated-imbibed	irradiated	irradiated-imbibed	irradiated	irradiated-imbibed	irradiated	irradiated-imbibed
A	13.68	21.14	15.80	12.75	10.23	23.36	20.32	20.36
B	26.28	31.07	25.05	25.01	22.31	30.39	37.97	33.28
C	23.78	20.76	23.69	23.33	25.87	20.07	23.56	23.30
D	18.11	14.06	18.23	20.51	22.42	14.28	11.91	13.88
E	18.15	12.97	17.23	18.40	19.16	11.91	6.24	9.17
total	100	100	100	100	100	100	100	100
% improvement		54.53		none		128.35		0.20

FIGURE 3.1 The correlation between the RNA/DNA ratios and the germination in the cold stress test of seedlots from cvs. Marathon, Matador and Cyrano (seedlots 1 experiment).

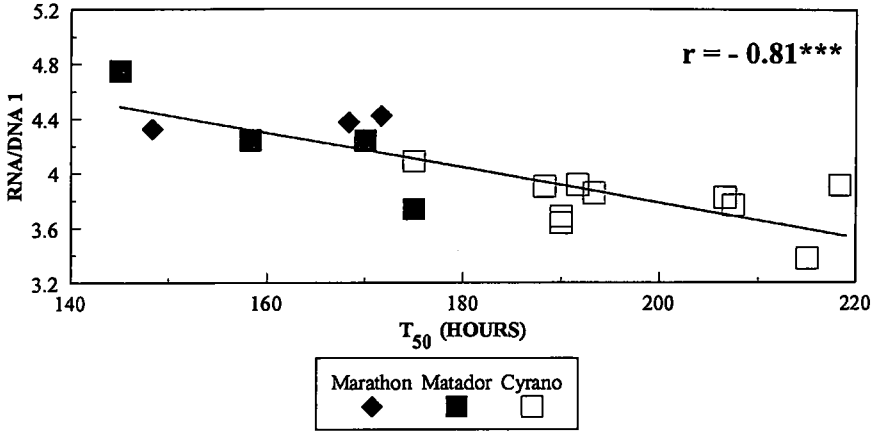
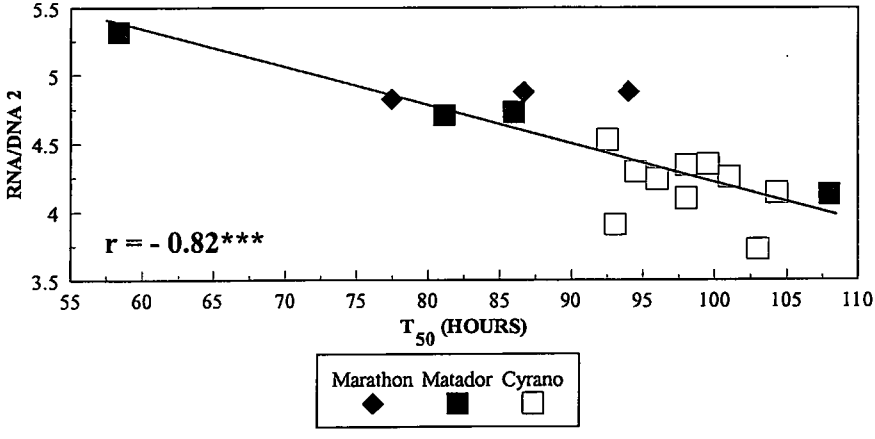


FIGURE 3.2 The correlation between the RNA/DNA ratios and the germination at 15°C of seedlots from cvs. Marathon, Matador and Cyrano (seedlots 1 experiment).



For Figures where correlation analyses are presented, axes do not indicate dependency.

FIGURE 3.3 The correlation between the RNA/DNA ratios and the germination on day 4 of the wet stress test of seedlots from cvs. Marathon, Matador and Cyrano (seedlots 1 experiment).

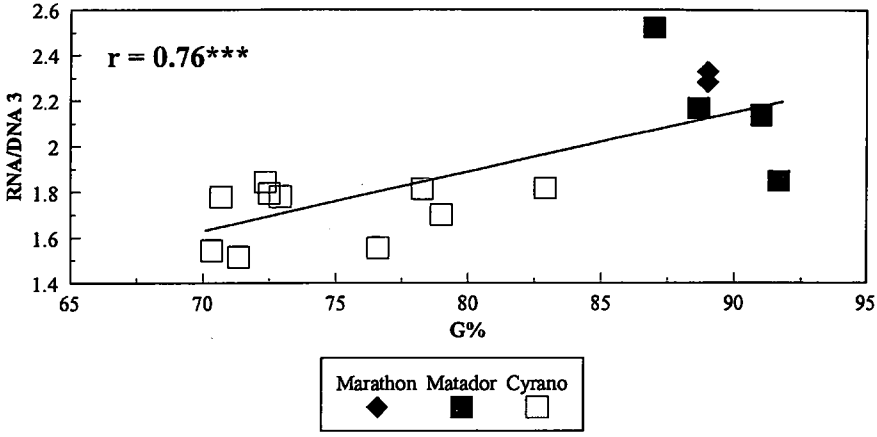


FIGURE 3.4 The correlation between the RNA/DNA ratios and the germination at 15°C of seedlots from cvs. Marathon, Matador and Cyrano (dotted line) compared to the correlation without cv. Cyrano seedlots (black line).

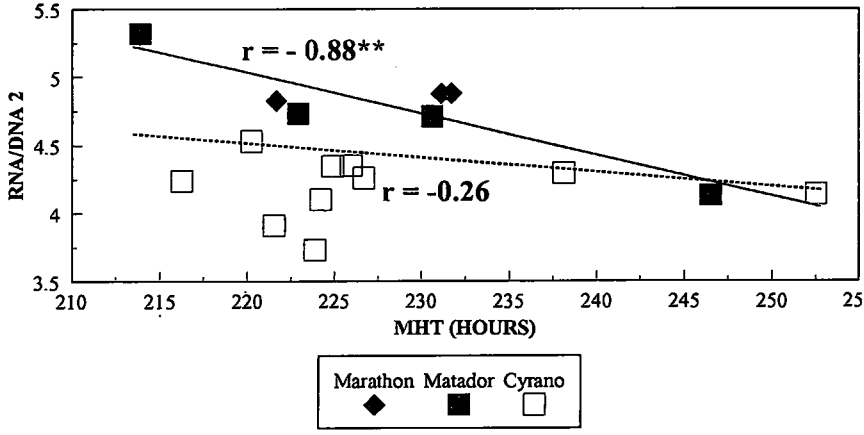


FIGURE 3.5 The correlation between the RNA/DNA ratios and the germination on day 3 at 20° C of the seedlots from cv. Rizor.

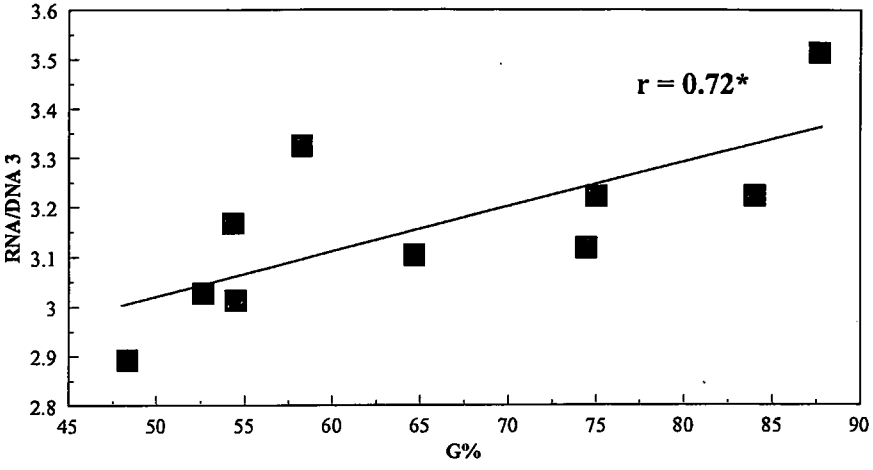


FIGURE 3.6 Germination of treated seeds at 9° C.

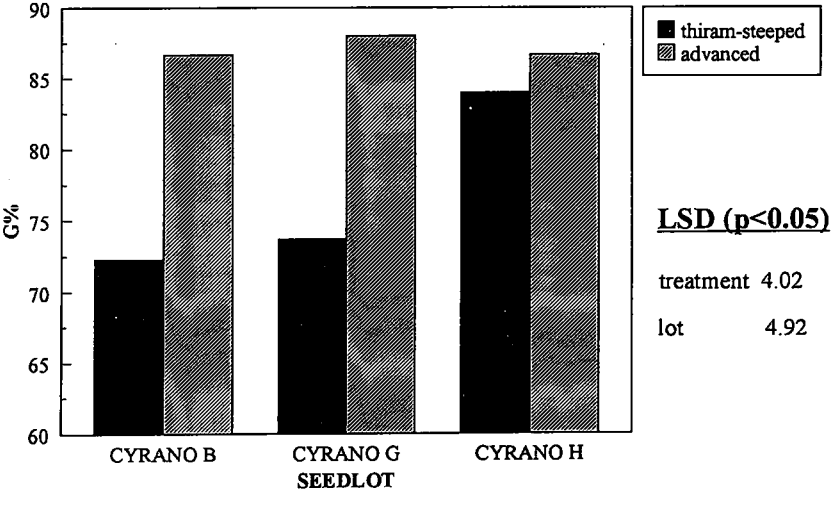


FIGURE 3.7 Germination of treated seeds at 9°C.

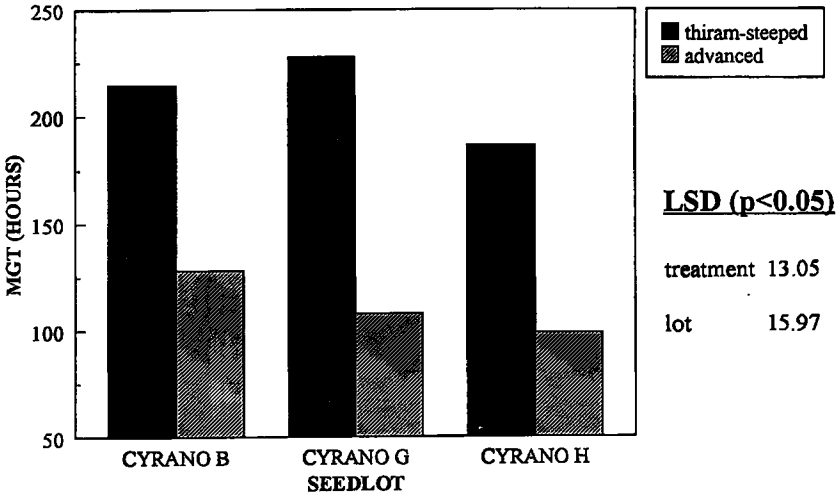


FIGURE 3.8 Germination of treated seeds at 9°C

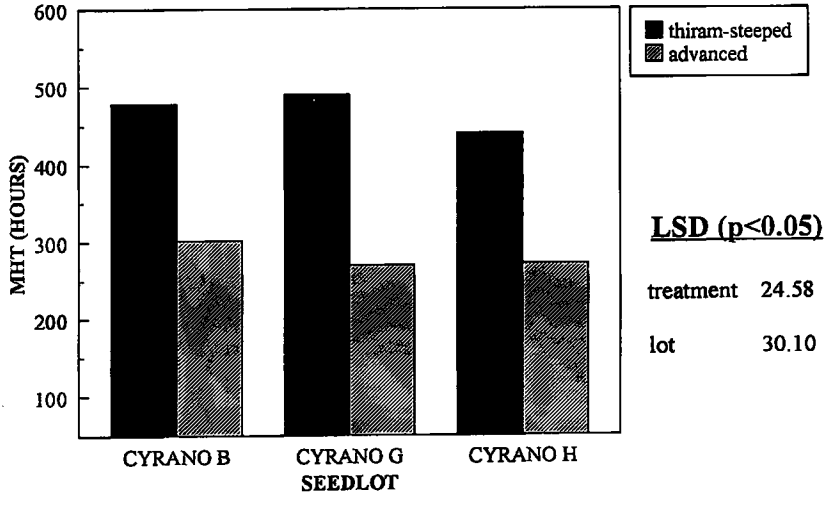


FIGURE 3.9 The correlation between the RNA/DNA ratios and the germination at 9 °C of the treated seedlots, cv. Cyrano B, G and H.

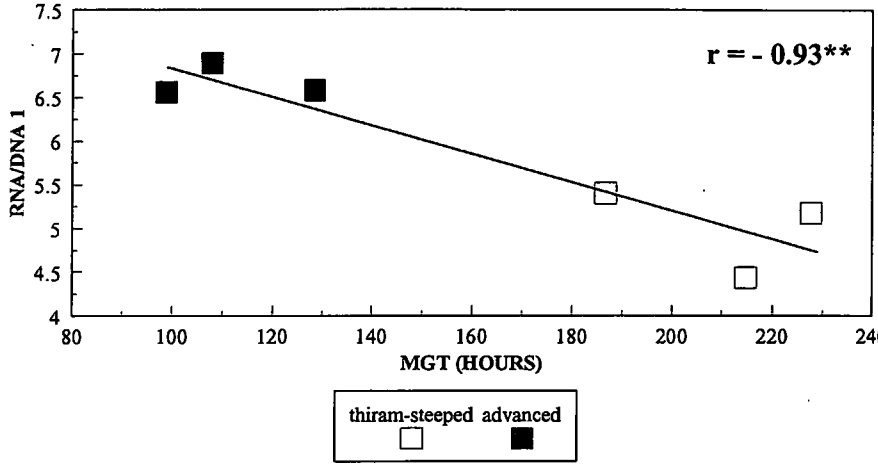


FIGURE 3.10 The correlation between the RNA/DNA ratios and the germination on day 2 of the wet stress test of selected seedlots from cvs. Rizor, Matador and Cyrano (seedlots 2 experiment).

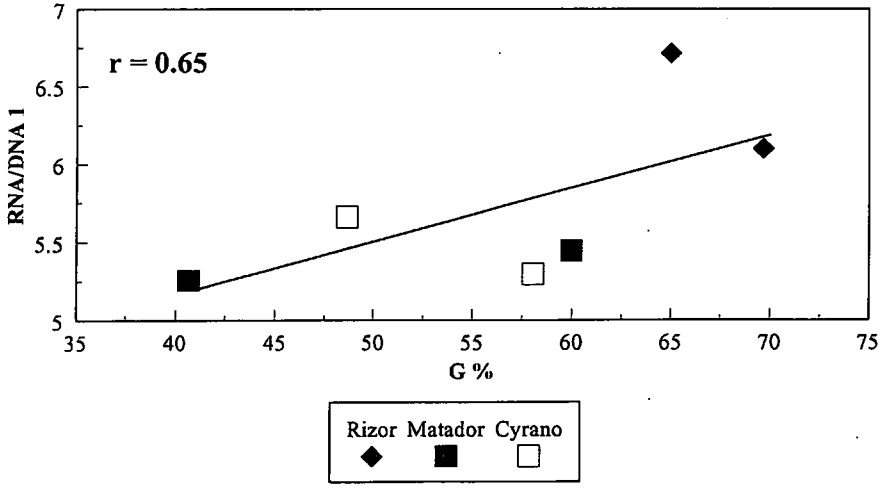


FIGURE 3.11 The correlation between the RNA/DNA ratios and the germination in the cold stress test of selected seedlots from cvs. Rizor, Matador and Cyrano (seedlots 2 experiment).

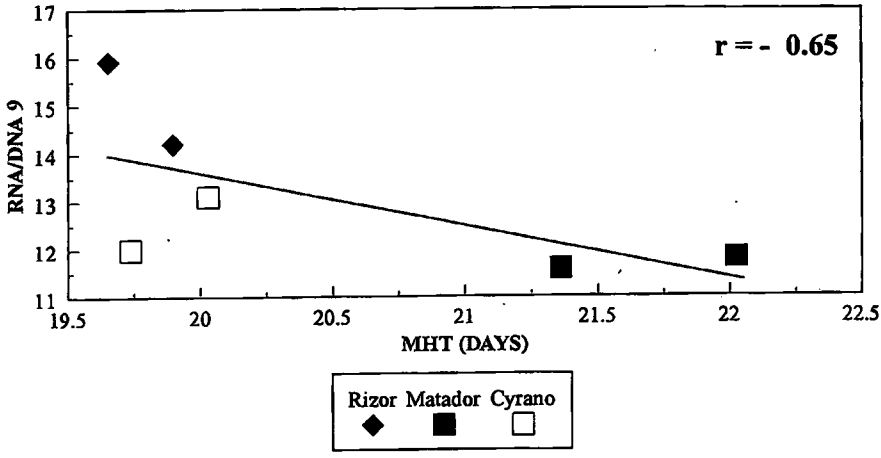


FIGURE 3.12 The correlation between the RNA/DNA ratios and T_{50} in the cold stress test of selected seedlots from cvs. Rizor, Matador and Cyrano (seedlots 2 experiment).

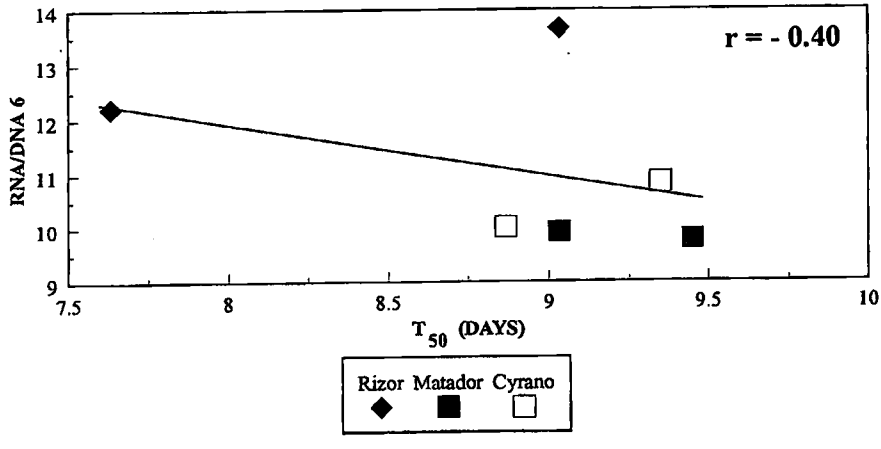


FIGURE 3.13 The correlation between the RNA/DNA ratios and establishment in field trial 1 of seedlots from cvs. Marathon, Matador and Cyrano (seedlots 1 experiment).

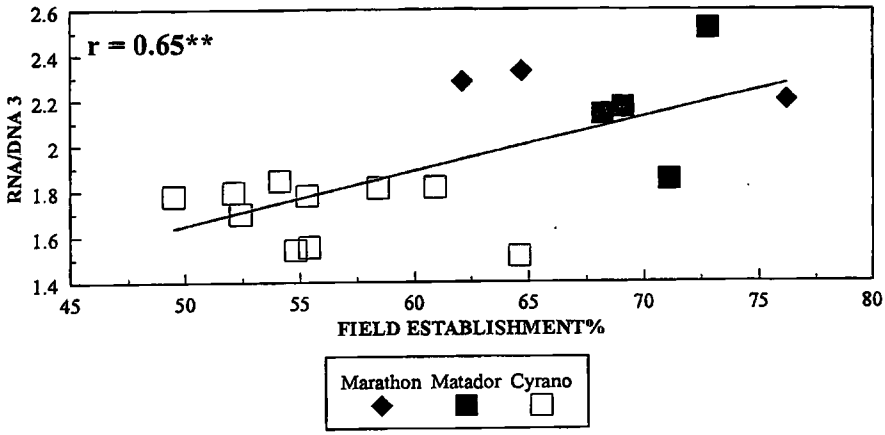


FIGURE 3.14 The correlation between the RNA/DNA ratios and emergence in field trial 1 of seedlots from cvs. Marathon, Matador and Cyrano (seedlots 1 experiment).

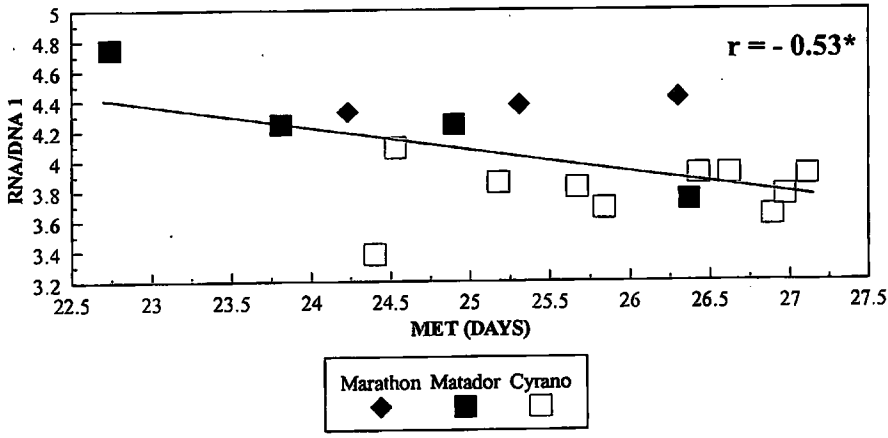


FIGURE 3.15 The correlation between the RNA/DNA ratios and emergence in field trial 1 of seedlots from cvs. Marathon, Matador and Cyrano (seedlots 1 experiment).

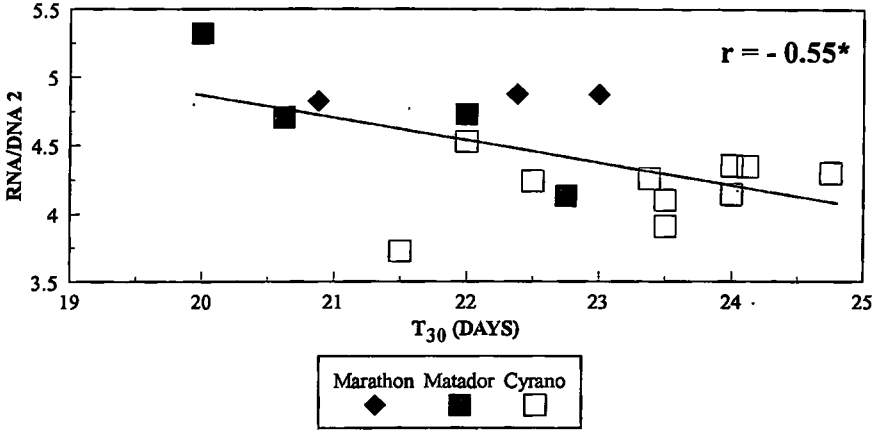


FIGURE 3.16 The correlation between germination in the cold stress test and establishment in field trial 1 of seedlots from cvs. Marathon, Matador and Cyrano (seedlots 1 experiment).

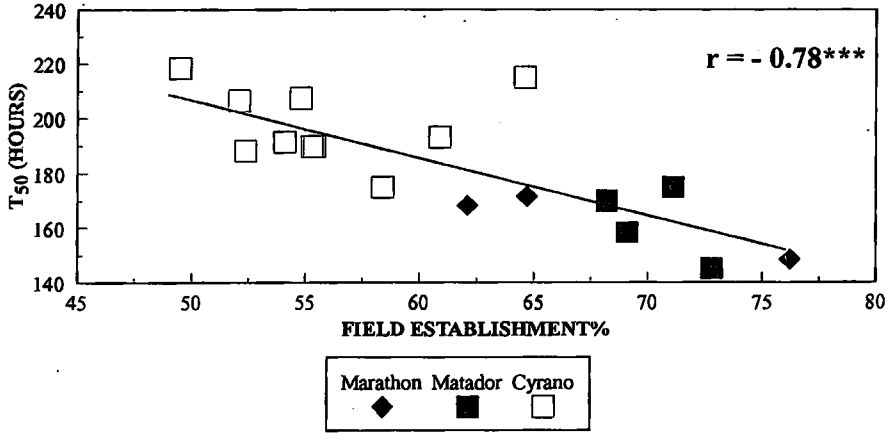


FIGURE 3.17 The effect of seed treatments on the field emergence of seedlots from cvs. Cyrano and Matador.

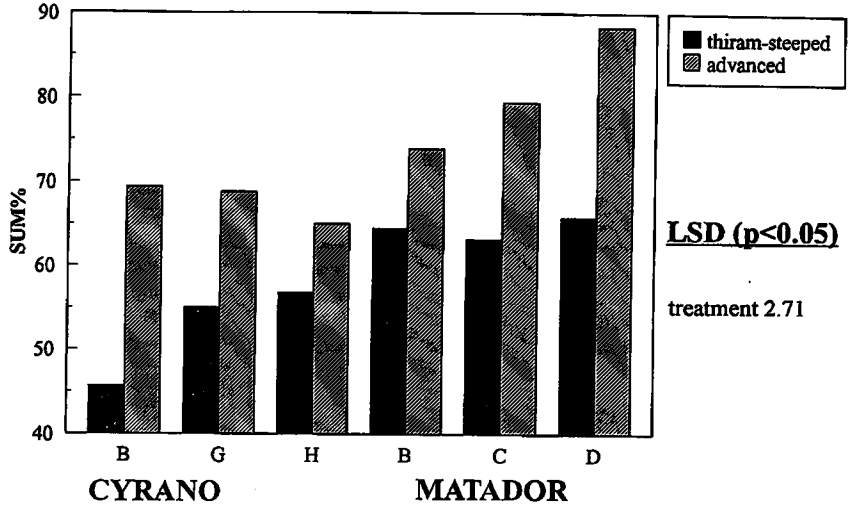


FIGURE 3.18 The effect of seed treatments on the field establishment of seedlots of cvs. Cyrano and Matador.

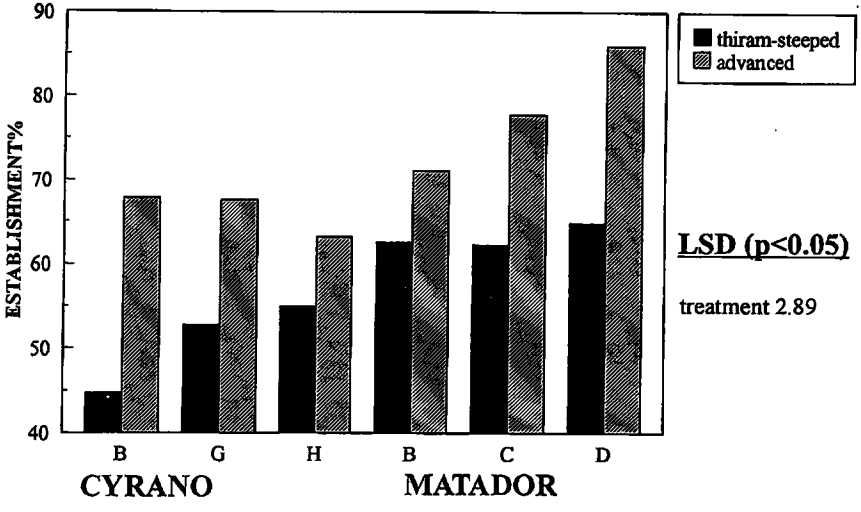


FIGURE 3.19 The effect of seed treatments on the field emergence of seedlots from cvs. Cyrano and Matador.

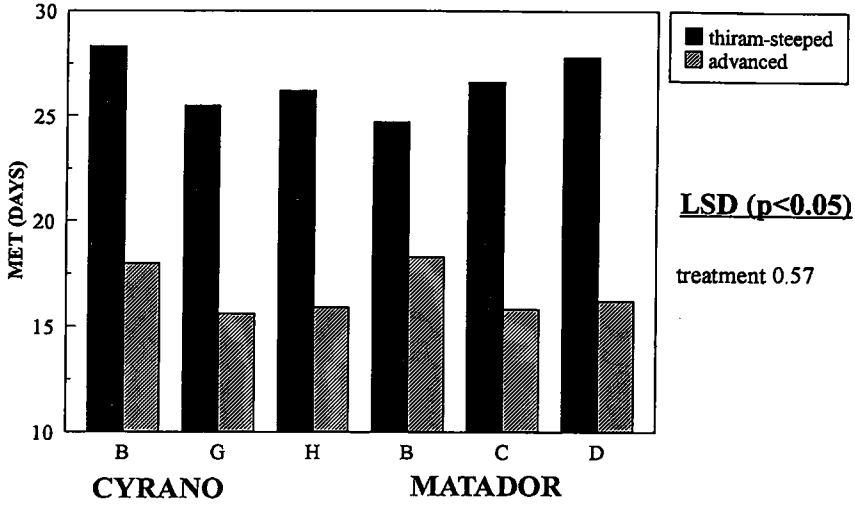


FIGURE 3.20 The effect of seed treatments on the correlation between the RNA/DNA ratios and field establishment of the treated seedlots, cv. Cyrano B, G and H.

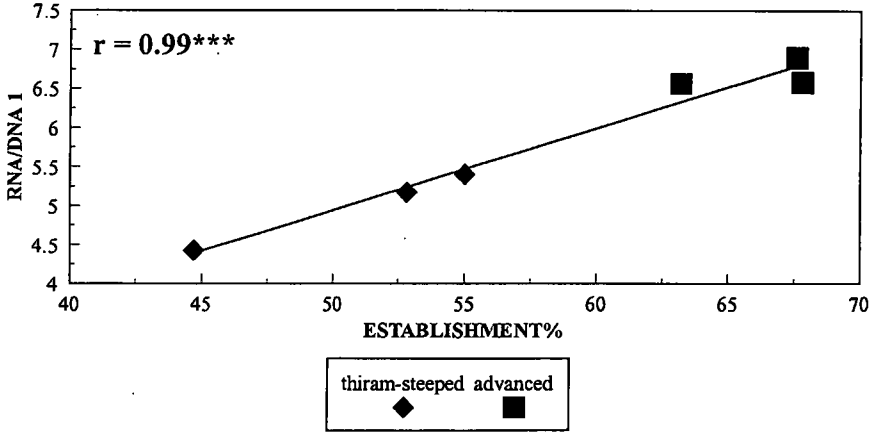


FIGURE 3.21 The effect of seed treatments on the correlation between the RNA/DNA ratios and field emergence of the treated seedlots of cv. Cyrano B, G and H.

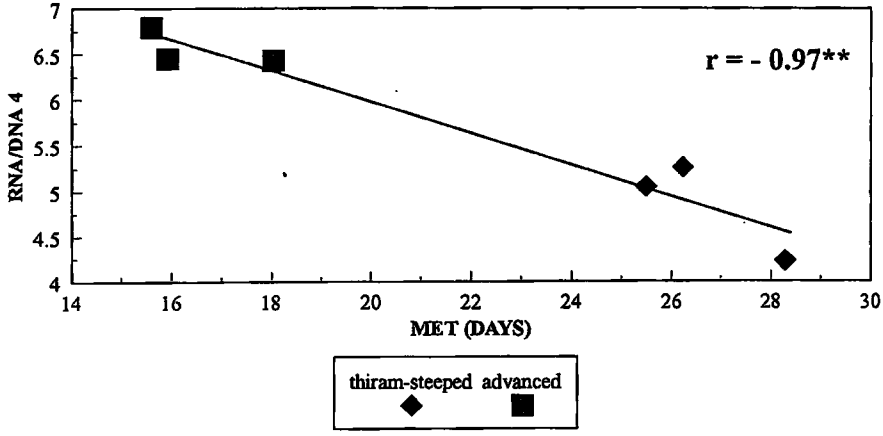


FIGURE 3.22 The effect of seed treatments on the correlation between the MGT in the cold stress test and field establishment of the treated seedlots cv. Cyrano B, G and H.

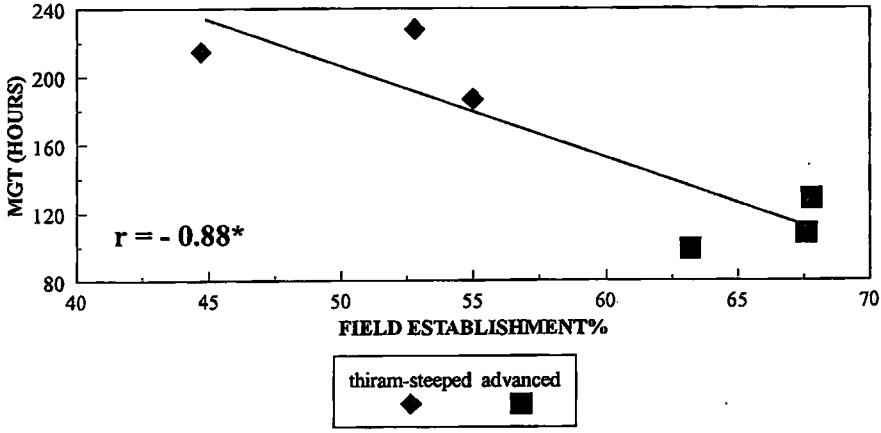


FIGURE 3.23 The effect of seed treatments on the correlation between germination on day 3 at 20°C and field emergence of the treated seedlots, cv. Cyrano B, G and H.

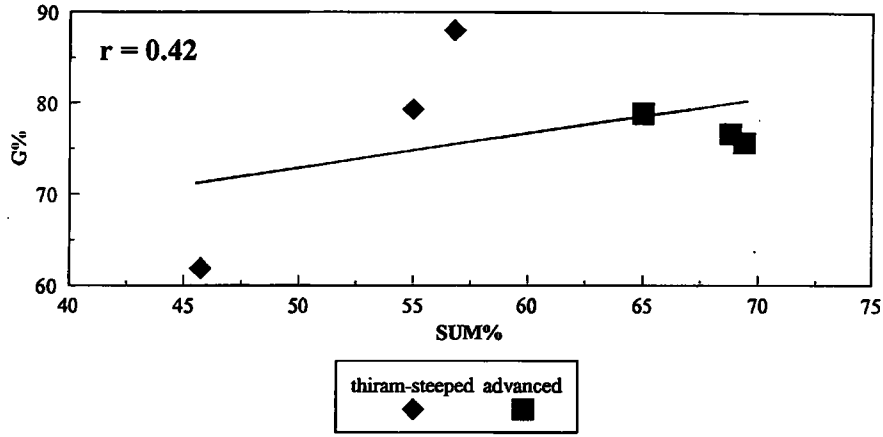


FIGURE 3.24 The effect of the seed treatments, rubbing and density-grading, on emergence in the field of cv. Planet.

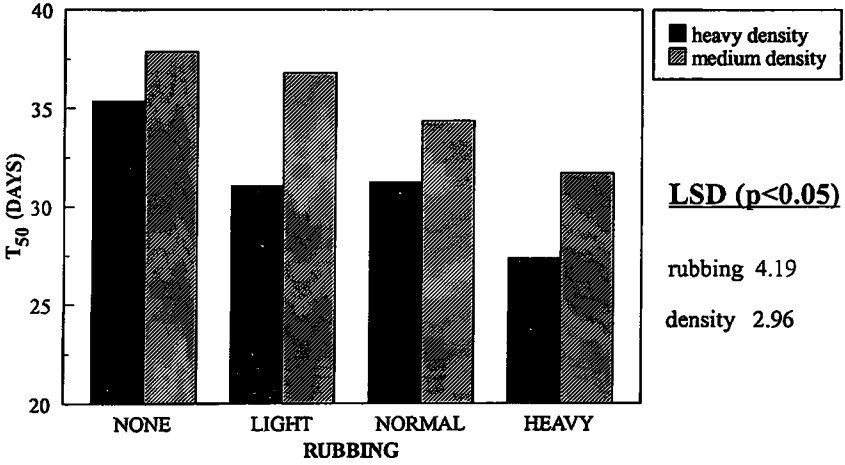


FIGURE 3.25 The correlation of the RNA/DNA ratios with the emergence in the field of seedlots from cv. Rizor.

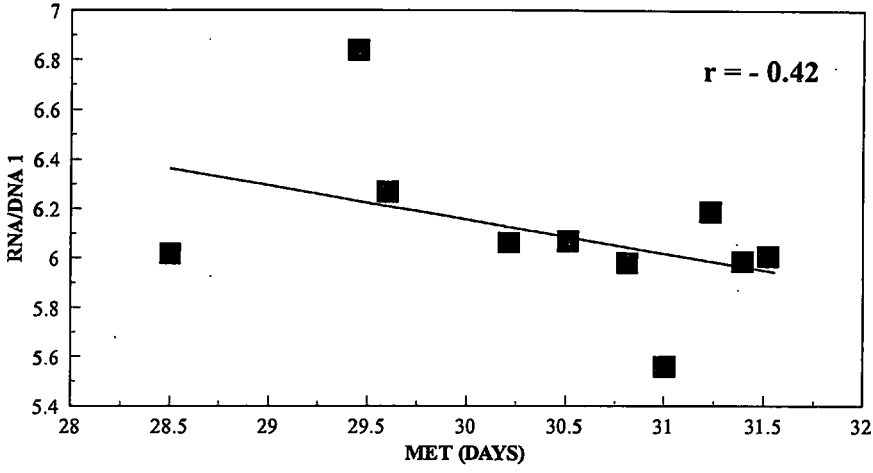


FIGURE 3.26 The correlation of the seedling development of cv. Rizor seedlots on day 6 of the germination test at 20 °C with field emergence.

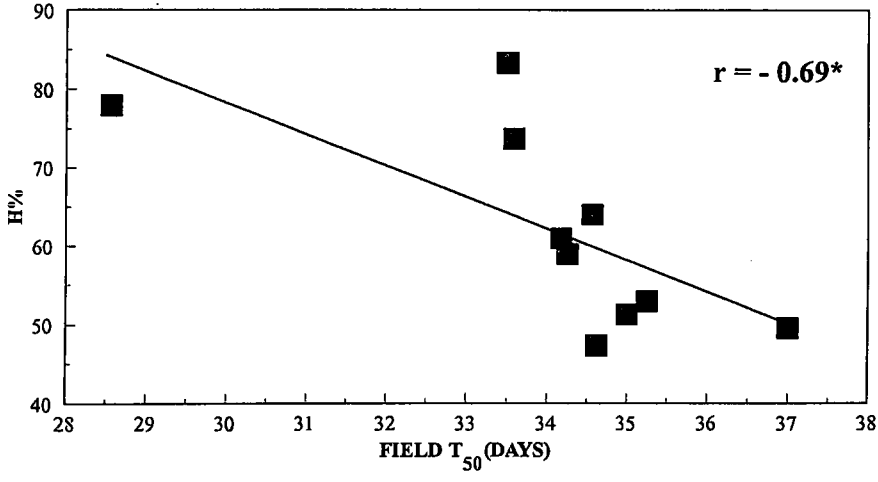


FIGURE 3.27 The correlation of the germination of seedlots from cv. Rizor in the cold sand test with rate of field emergence.

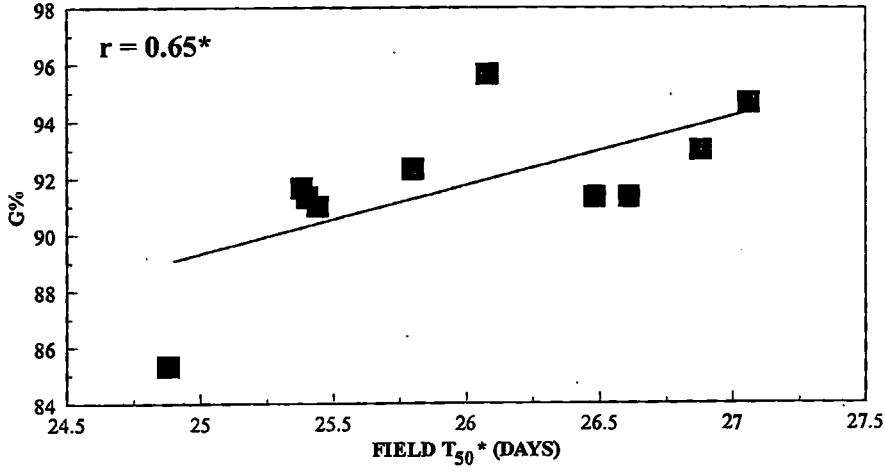


FIGURE 3.28 The correlation between the RNA/DNA ratios and field establishment of selected seedlots from cvs. Cyrano, Rizor and Matador (seedlots 2 experiment).

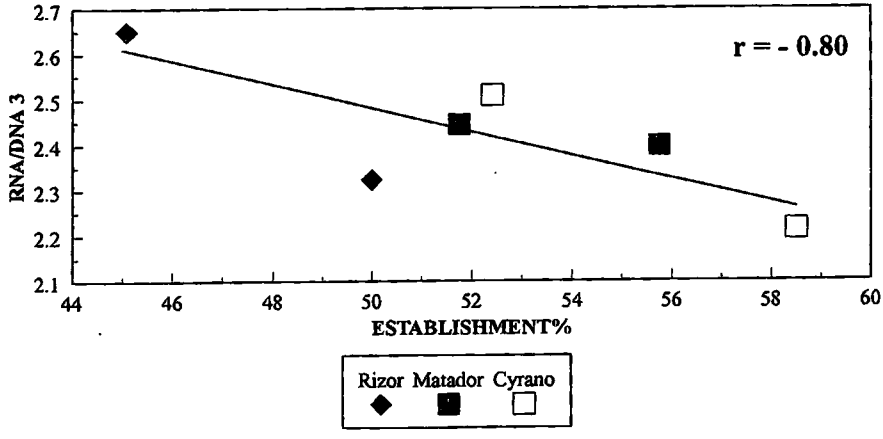


FIGURE 3.29 The correlation between the RNA/DNA ratios and field emergence of selected seedlots from cvs. Cyrano, Rizor and Matador (seedlots 2 experiment).

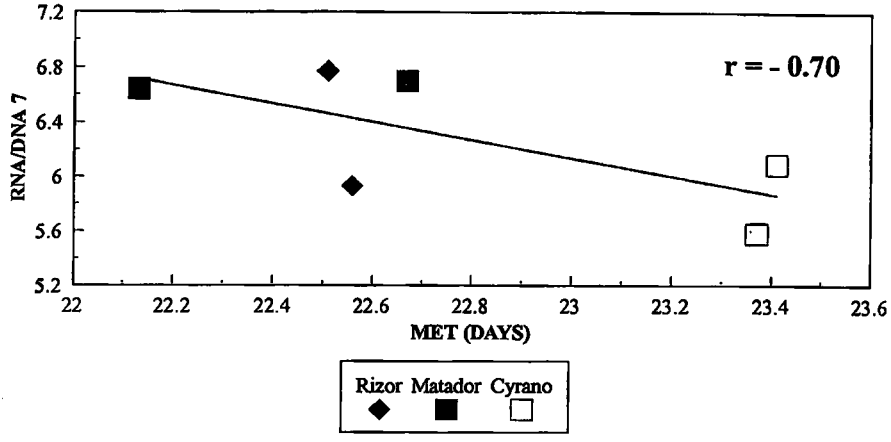


FIGURE 3.30 The correlation between the RNA/DNA ratios and total seedling dry weight per plot of selected seedlots from cvs. Cyrano, Rizor and Matador (seedlots 2 experiment).

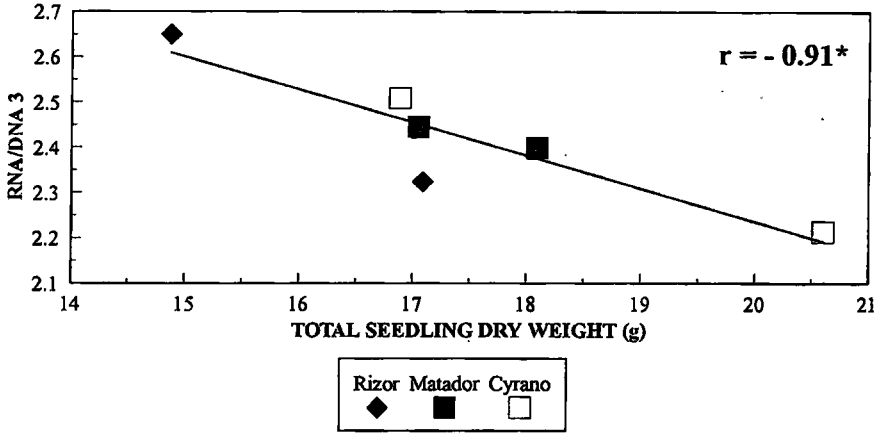


FIGURE 3.31 The correlation between field establishment and total seedling dry weight per plot of selected seedlots from cvs. Cyrano, Rizor and Matador (seedlots 2 experiment).

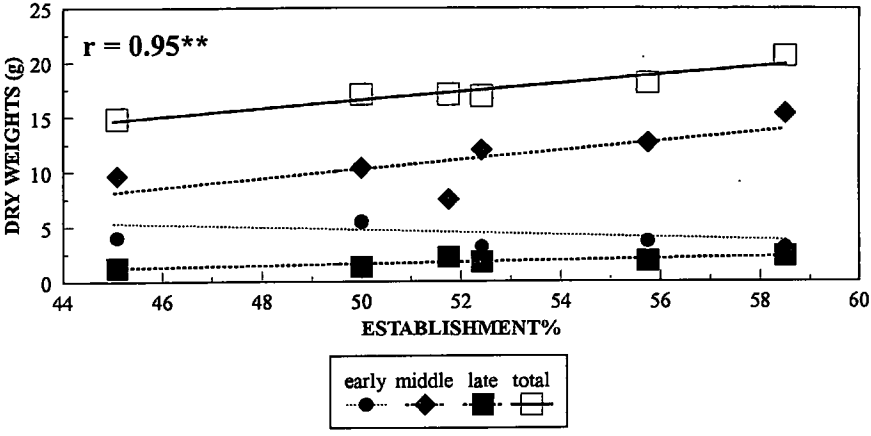


FIGURE 3.32 The correlation between field emergence and dry weight per plant of early emerging seedlings of selected seedlots from cvs. Cyrano, Rizor and Matador (seedlots 2 experiment).

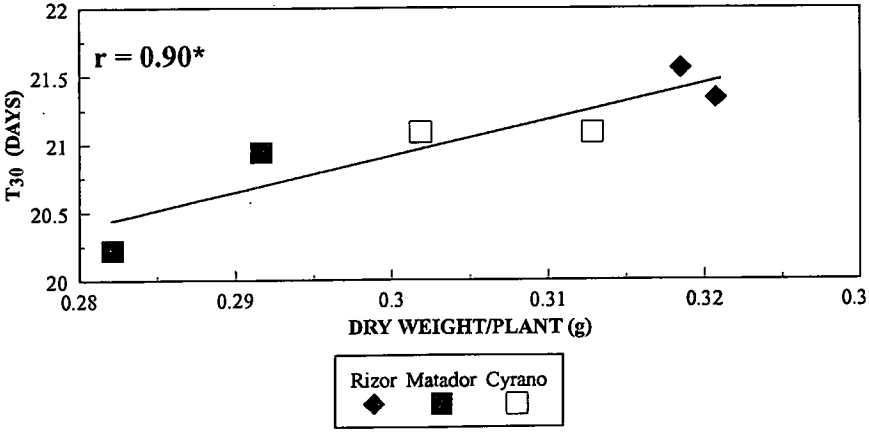


FIGURE 3.33 The correlation between germination in the cold stress test and field emergence of selected seedlots from cvs. Cyrano, Rizor and Matador (seedlots 2 experiment).

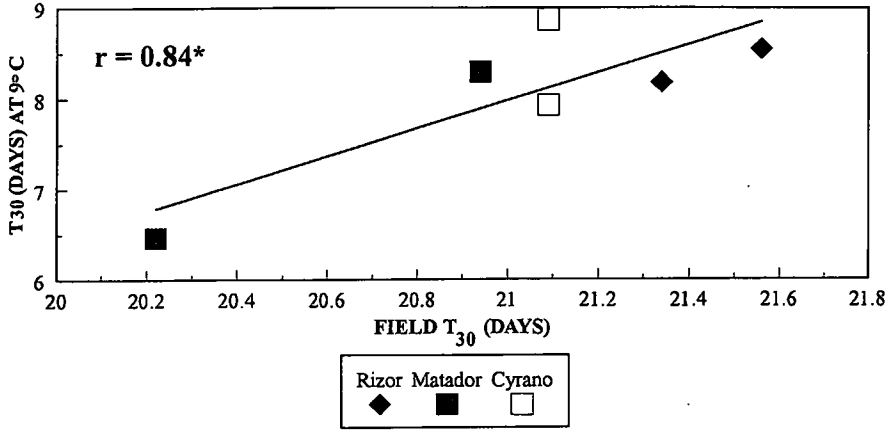


FIGURE 3.34 The effect of fruit size on the RNA/DNA ratio.

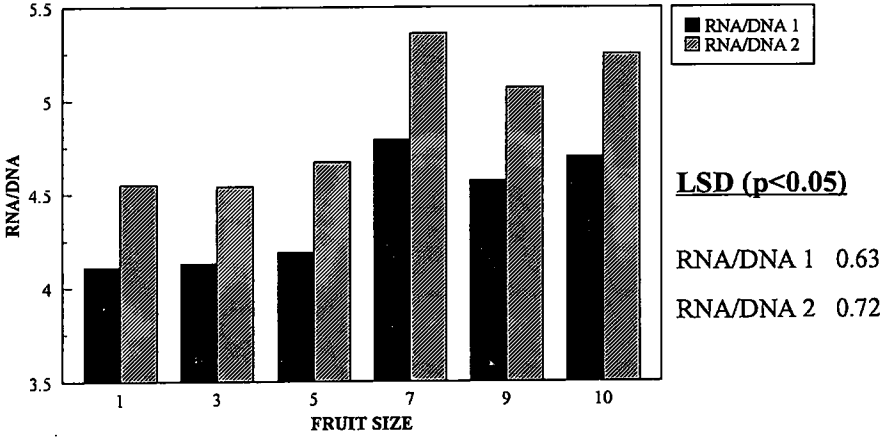


FIGURE 3.35 The correlation between the RNA/DNA ratios and the field emergence of size-graded fruits (size grades 1, 3, 5, 7, 9 and 10).

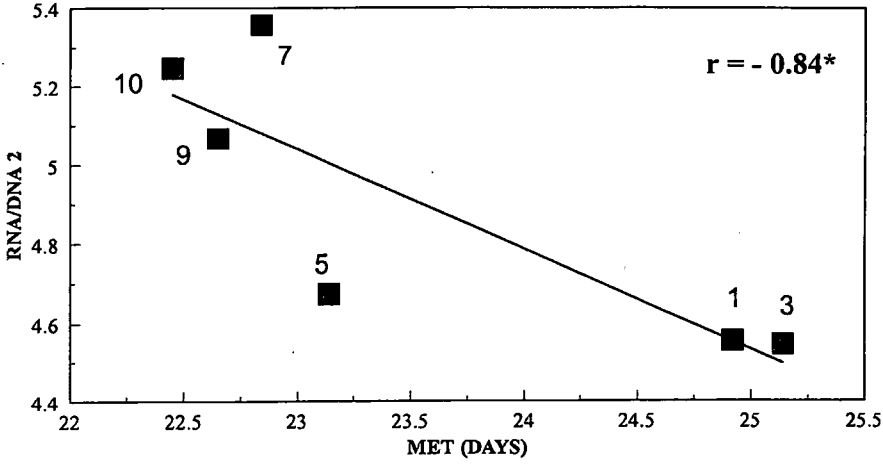


FIGURE 3.36 The effect of ploidy on the RNA/DNA ratio.

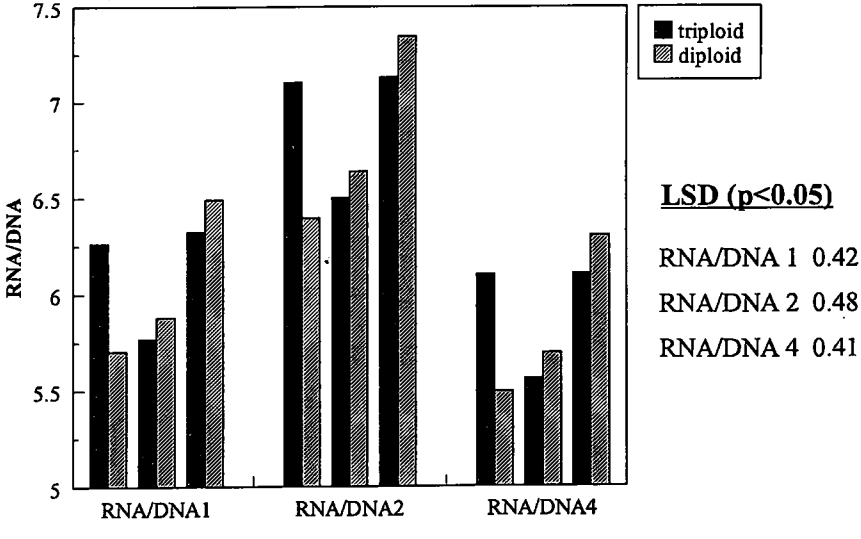


FIGURE 3.37 The effect of seed treatments on the RNA/DNA ratios of cv. Cyrano B, G and H seedlots.

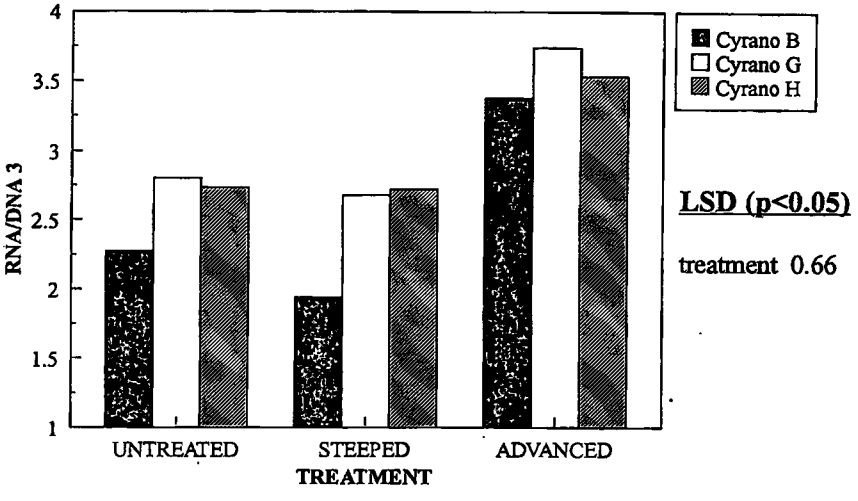


FIGURE 3.38 The effect of seed treatments on the RNA/DNA ratios of cv. Cyrano H seedlot.

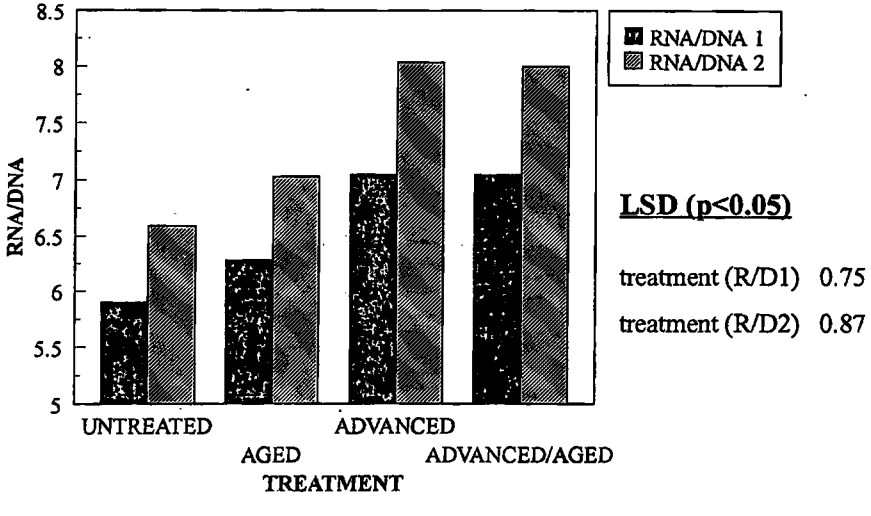


FIGURE 3.39 Feulgen staining of onion root tip cells.

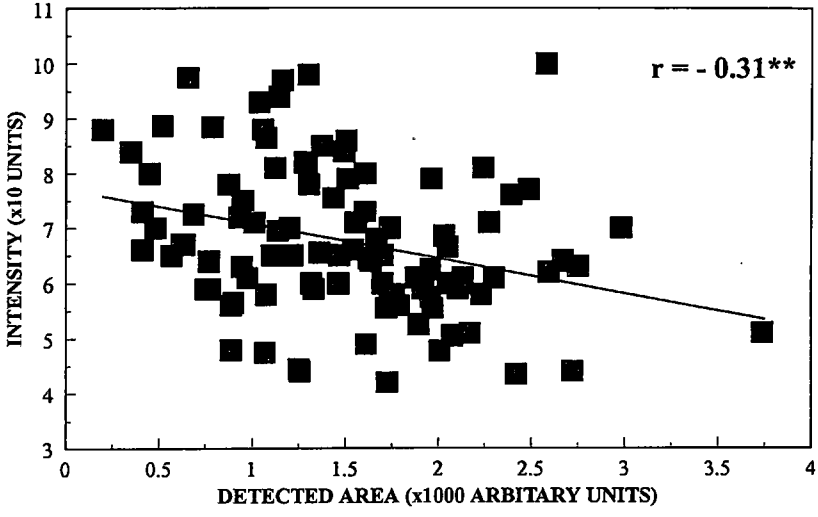


FIGURE 3.40 The DNA contents of Feulgen stained nuclei from different plant tissues.

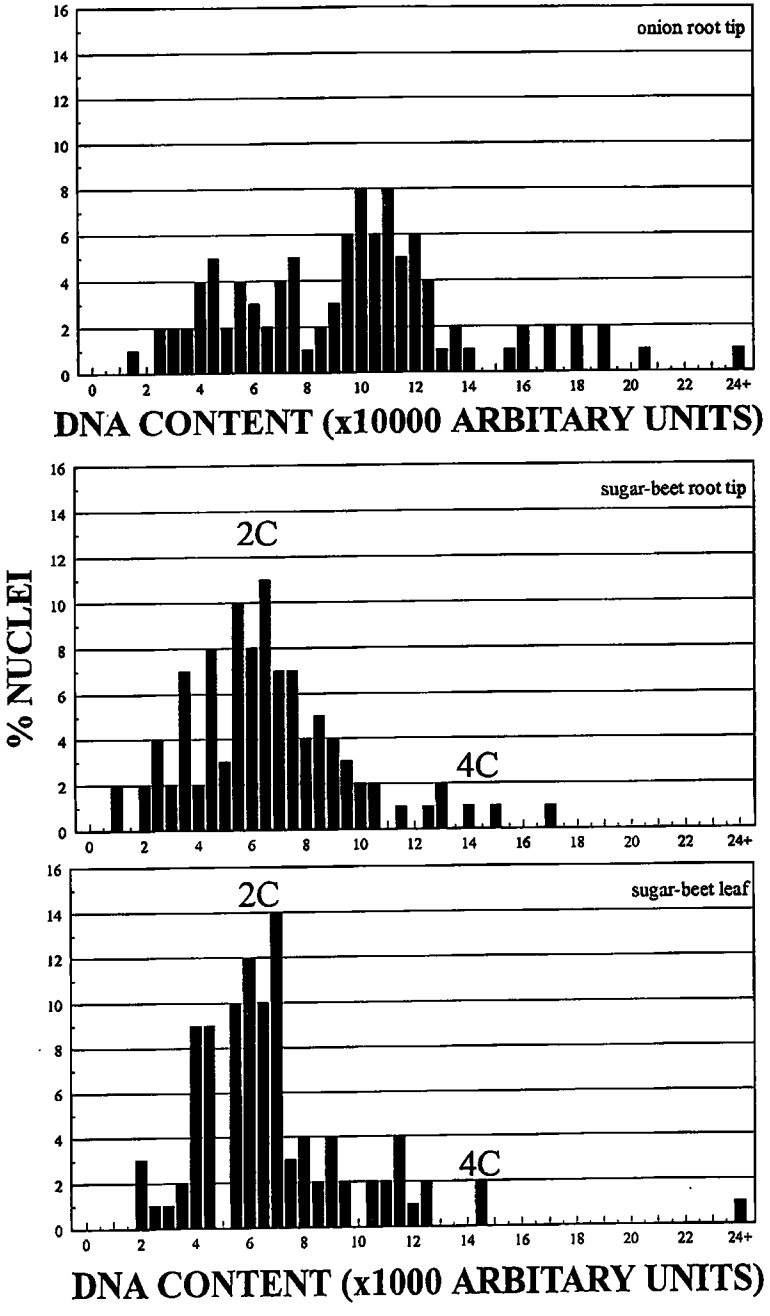


FIGURE 3.41 The effect of seed treatments on the DNA contents of cv. Cyrano G root tip cells.

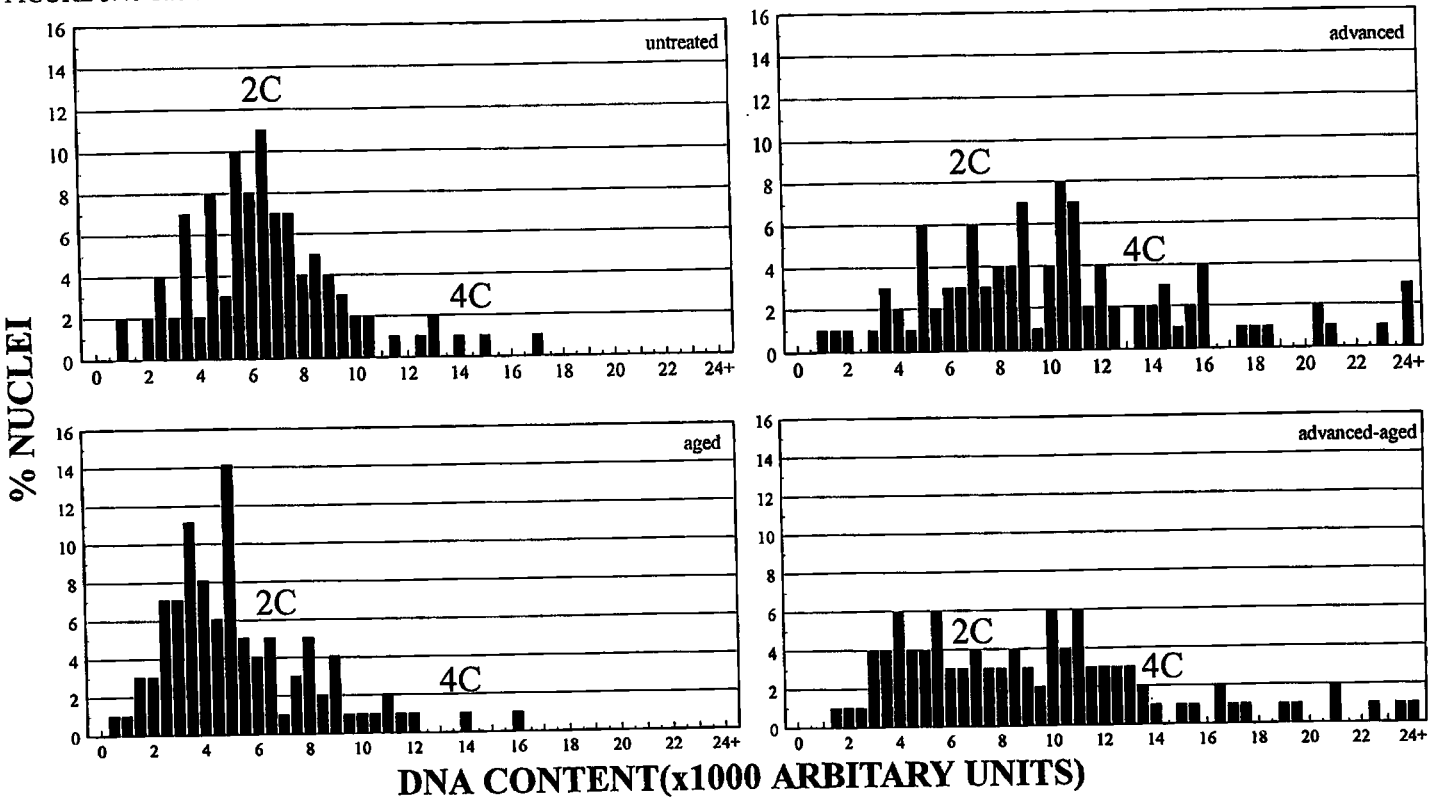


FIGURE 3.42 The DNA contents of root tip cells from the seeds of three cv. Cyrano seedlots.

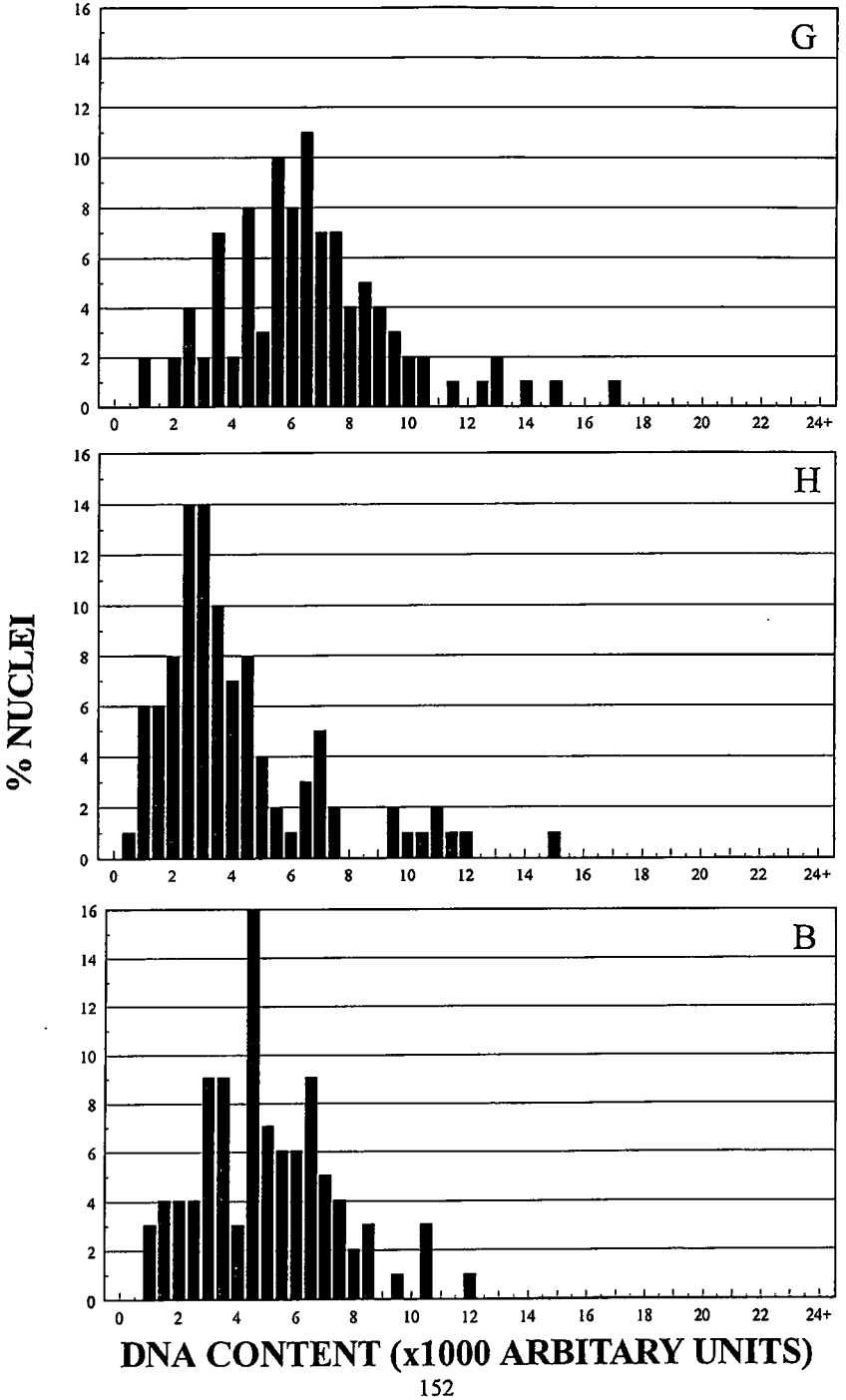


FIGURE 3.43 DNA samples from treated cv. Cyrano C embryos run on an alkaline gel overnight (lanes 1=control; 2=irradiated; 3=irradiated then imbibed at 24°C for two hours).

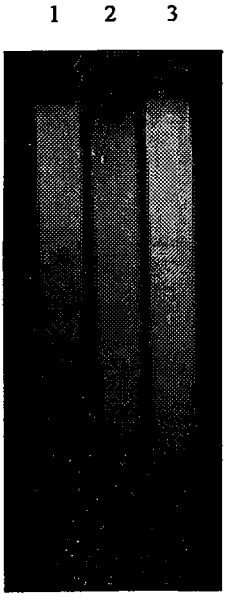
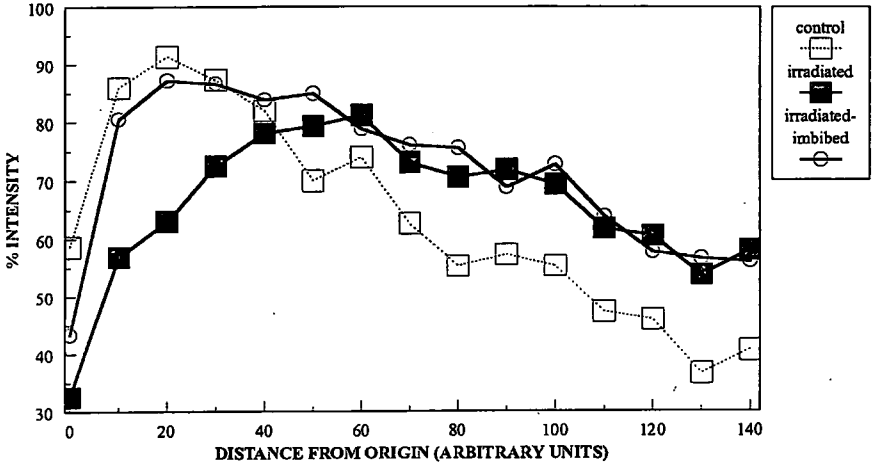


FIGURE 3.44 The DNA fragmentation profiles of the untreated, irradiated and then imbibed embryos run on an alkaline gel.



4.1. LABORATORY ASSESSMENT

In this section, the results of each type of germination test and the RNA/DNA ratio will be discussed. The effect of vigour on germination test performance and the RNA/DNA ratio were determined in the seedlots 1 and seedlots 2 experiments. The effect of artificially improving vigour, using the primed advancement treatment instead of thiram-steeping, on the laboratory performance of the seedlots will also be discussed.

4.1.1. **Seedlots 1 experiment**

When the seventeen seedlots of three cultivars (cvs. Cyrano, Marathon and Matador) were assessed, in general the RNA/DNA ratios, calculated in three different ways, correlated significantly with seed vigour assessed using germination tests (Table 3.1). The RNA/DNA ratios also ranked the seedlots in the same order as most of the germination tests. The seedlots which germinated fastest at 9°C and 15°C, and could be said to be of high vigour, had larger RNA/DNA ratios. The seedlots which had larger germination percentages in the standard germination test at 20°C, in the cold stress test and in the wet stress test also had larger RNA/DNA ratios. However, in general, MHT at 9°C and 15°C did not correlate significantly with the RNA/DNA ratios or with results from the other germination tests. This also applied to the early H% count in the standard germination test at 20°C and the wet stress test. Measurements of hypocotyl growth may be more sensitive than radicle protrusion to the artificial conditions, such as low illumination, imposed during the germination tests.

When the seedlot assessment data were analysed for each cultivar individually (cvs. Cyrano, Marathon and Matador and Rizor), there were few significant correlations between the RNA/DNA ratios and the germination test results. This may be because intra-cultivar differences were not large enough to be detected using the RNA/DNA ratio. Interestingly, the significant correlations of cvs. Marathon and Matador seedlots between the RNA/DNA ratios and the germination tests (Table 3.3) were also significant when comparing different types of germination tests (data not shown). This is in contrast to the situation with the cv. Cyrano seedlots (Table 3.2) and the cv. Rizor seedlots (Table 3.4) which had a different pattern of

correlations between the RNA/DNA ratio and the germination tests and the germination tests compared to each other (data not shown). This might be explained by the differences between seedlots being too small to be assessed but when two or three cultivars were analysed together then the inter-cultivar differences were large enough to generate a linear relationship between the different laboratory tests. To test this hypothesis, two seedlots from each cultivar were selected based on showing a consistent performance in the laboratory and field tests (see seedlots 2 experiment in section 4.1.3.). This was to allow a more detailed study to see if the RNA/DNA ratio would consistently differentiate between the vigour of seedlots from different cultivars.

4.1.2. **Seedlots 2 experiment**

RNA/DNA ratios calculations 5 and 6 (section 2.2.3.3.2.) produced the same linear correlative relationship when they were plotted against the germination test measurements of the six seedlots (Table 3.9). The correlation coefficients were also the same for RNA/DNA calculation 7 with 8 and also 9 with 10. However, there were no significant correlations between the RNA/DNA ratios and the germination test measurements. The ranks assigned to each seedlot by the germination tests were also significantly different from the ranks generated by the RNA/DNA ratios. Although not significant, the RNA/DNA calculations 1, 7 and 8 generated the largest correlation coefficients with the germination test measurements. RNA/DNA calculation 1 does not take into account any measurement of contaminants in the DNA and RNA sample. RNA/DNA calculations 7 and 8 utilise a graph of DNA standards measured on the fluorimeter and on the spectrophotometer in order to estimate the amount of RNA in the total nucleic acid sample (see section 2.2.3.3.2.). RNA/DNA 7 and 8 also include the deduction of an estimate of contamination from the total nucleic acid solution. Also, $G\%_{wet,d2}$ compared with the RNA/DNA ratios generated the largest, but non-significant, correlation coefficients of the germination test measurements.

If the germination test measurements are compared against each other then the cold stress test measurements, in general, generate different relative values of the seedlots to the other germination tests (Table 3.10). The wet stress test measurements correlated significantly with the measurements in the standard germination test at 20°C and in the cold sand test. This may be explained by the cold stress test quantifying the rate of germination of each seedlot whereas

the other tests measured germination levels on specified days. Also in the wet stress test, standard germination test and for half of the time period of the cold sand test, the seeds experienced temperatures which were optimum in contrast to the cold stress test at 9°C.

4.1.3. **Steeping-advancing experiment**

The cv. Cyrano seedlots which underwent the advancement treatment had significantly higher RNA/DNA ratios than the thiram-steeped seeds. In addition, the advanced seeds also performed significantly better in the germination test at 9°C although this was not the case in the germination test at 20°C. This might be explained by the fact that only when the germination conditions are stressful ie. 9°C is there a measurable improvement in the germination performance of advanced seeds.

The RNA/DNA ratios of the treated seedlots were significantly correlated with the germination test measurements in the cold stress test (Table 3.8). The advanced seeds which germinated more quickly also had larger RNA/DNA ratios. The changes in nucleic acid levels following seed treatments will be discussed in 4.3.3.

One of the aims of seed treatments is to increase the synchrony of germination. In addition to improving the rate of germination, the advancement treatment produced a germination level which was more uniform between the seedlots.

4.2. FIELD AND LABORATORY ASSESSMENT

4.2.1. **Field trial 1**

The seedlots from cultivars, Cyrano, Marathon and Matador were assessed for rate of emergence (T_{50}, T_{50}*, T_{30} and MET) and number of emerged seedlings at establishment (sum% and establishment%). Sum% is the summation of the daily counts up until the 4-6 true leaf stage which is the same as the establishment count except that the sum% does not take in to account post-emergence losses. T_{50} was not analysed by ANOVA because certain seedlots did not reach 50% emergence.There were significantly greater differences between seedlots of different cultivars than between seedlots of the same cultivar for sum%, establishment% and

T_{30}. This would be expected because seedlots from the same cultivar have a more similar genetic makeup than seedlots from different cultivars. MET and T_{50}* did not show significantly larger inter-cultivar differences than intra-cultivar differences, which may have been due to the values of MET and T_{50}* being very similar for all the seedlots.

4.2.1.1. Relationships between the RNA/DNA ratios and field trial measurements

There were significant correlations for the field MET, sum%, establishment% and T_{30} with all three RNA/DNA ratio calculations when the seedlots from cvs. Cyrano, Marathon and Matador were analysed (Table 3.11). For example, the seedlots from cv. Cyrano, which showed low establishment in the field and on average took longer to emerge (MET and T_{30}), had lower RNA/DNA ratios in comparison with cvs. Marathon and Matador. Only RNA/DNA ratio 3, which includes an estimate of contamination, correlated significantly (Pearson's correlation) with field T_{50}. When the seedlots were ranked (using Spearman's rank correlation analysis) in terms of their RNA/DNA ratios and field performance, RNA/DNA ratios 1 and 2 had significant rank correlation coefficients with T_{50}. This means that the rankings generated by the RNA/DNA calculations 1 and 2 were similar to the rankings generated by rate of emergence in the field but this relationship was not linear because the Pearson's correlation coefficient was not significant.

When only the seedlots from cv. Cyrano were analysed, there were no significant correlations between the RNA/DNA ratios and field performance measurements (Table 3.12). This indicates that again the RNA/DNA ratio is better at differentiating between seedlots of different cultivars but not between seedlots of the same cultivar. Similarly, when the seedlots of cvs. Marathon and Matador were analysed together (Table 3.13) there were no significant correlations between the RNA/DNA ratios and field measurements which may be explained by the seedlots of both cultivars being similar in terms of vigour levels. However, RNA/DNA ratios (1 to 3) showed large, but non-significant, correlation coefficients when they were compared with rates of emergence in the field (MET, T_{50}* and T_{30}).

4.2.1.2. Relationships between the germination test results and field trial measurements

The germination test results of the seedlots from the three cultivars were compared with field

performance (Table 3.14). In particular, the T_{50} and MGT measurements from the standard germination tests at 9°C and 15°C correlated significantly with all the field trial measurements, as did early germination levels in the test at 20°C and $H\%_{wet,d4}$. These germination tests have in common the fact that they are measuring early germination or rate of germination.

In the same way as for the RNA/DNA ratios, if the results of the seedlots from cv. Cyrano were analysed on their own (Table 3.15), then there were few significant correlations between germination test measurements and field performance. The only germination test measurement which correlated significantly with most of the field trial measurements was the $H\%_{wet,d4}$. However, MHT at 15°C had large, but non-significant, correlation coefficients when compared with rates of emergence in the field. Two measurements from the standard germination test ($G\%_{20C,d3}$ and $H\%_{20C,d6}$) also correlated (not significantly) with sum% and establishment%. The latter measurement also correlated significantly ($p<0.05$) with T_{50}.

When the data for seedlots from cvs. Marathon and Matador were analysed it was found that no germination test measurements correlated significantly with field sum% and establishment% (Table 3.16). Measurements of rate of emergence in the field were significantly correlated with T_{50} and MGT in the cold stress test and early germination in the standard germination test at 20°C. Interestingly, there were positive correlations between MET and T_{50}* in the field and two germination test measurements ($H\%_{20C,d14}$ and G% on day 14 of the cold sand test), where negative linear relationships would have been expected. That is, it would have been expected for seedlots which emerge more quickly in the field and therefore have higher vigour, to perform better in germination tests than the other seedlots of lower vigour.

Therefore it appears that in general the germination tests and RNA/DNA ratios estimate field performance at the cultivar level but are not able to differentiate consistently between the seedlots of each cultivar possibly because the seedlots are very similar.

4.2.2. **Field trial 2**

For field trial 2, seedlots from cvs. Cyrano and Matador were thiram-steeped or advanced. The advancement treatment significantly improved field performance measured by sum%, establishment% and MET although it did not improve the field performance of each seedlot

to the same extent nor did it bring the seedlots up to the same level as each other. This is in contrast to the effect of advancement on seed germination in the standard test at 9°C where the advanced seeds showed a more similar germination percentage than the thiram-steeped seeds. This might be an indication of the variability in emergence generated in the field by a wide range of stresses and variables in comparison with the relatively controlled conditions in laboratory tests.

As in field trial 1, T_{50} data could not be analysed because some of the thiram-steeped seedlots did not reach 50% emergence. In addition, there were significantly larger inter-cultivar differences than intra-cultivar differences for sum% and establishment% but not for MET. In agreement with data from field trial 1, MET seems to be similar for all of the thiram-steeped seedlots independent of which cultivar the seedlot was from.

4.2.2.1. Relationships between the RNA/DNA ratios and field trial measurements

All four RNA/DNA ratio calculations correlated significantly with all of the field performance measurements for the treated cv. Cyrano seedlots (Table 3.17). Positive correlations were seen between seedling number at establishment and the RNA/DNA ratios. This indicates that advanced seeds, which had more seedlings at establishment than the thiram-steeped seeds, also had higher RNA/DNA ratios. Also, as might be expected, negative correlations were seen between estimates of rate of emergence in the field and RNA/DNA ratios. Advanced seeds emerged more quickly in the field than thiram-steeped seeds and also had larger RNA/DNA ratios.

4.2.2.2. Relationships between the germination test results and field trial measurements

The cold stress test results of the advanced and thiram-steeped seedlots correlated significantly with the field trial measurements (Table 3.18). Advanced seedlots on average germinated more quickly at 9°C and showed improved performance in the field. As noted in section 4.1.3., the advancement treatment did not have any affect on germination at 20°C on three different counting days. The fact that differences can only be seen between treatments in the field and in the germination test at 9°C, supports the explanation that conditions need to be stressful in order to produce a noticeable response to advancement.

In summary, the advancement treatment artificially improved vigour and this improvement could be consistently measured in the field and in the germination test at 9°C and was reflected in higher RNA/DNA ratios.

4.2.3. **Field trial 3**.

4.2.3.1. The effect of rubbing and grading on the field performance of cv. Planet

The seeds of cv. Planet were divided into four different lots based on the degree of rubbing to remove the seed cortex. The seeds in each lot were then graded in to heavy, medium or light density fractions. The lightest fraction was discarded in each case on account of them containing too many empty seeds.

Seeds with a high density showed higher establishment percentages in the field than those of medium density and they also emerged more quickly (Appendix E3). High density may be linked with seed quality because it as been shown that larger fruits tend to be more mature and are more likely to germinate (Hogaboam and Snyder 1964, Wood *et al.* 1977). This will be discussed in more detail in section 4.3.1.

In addition, a greater degree of rubbing increased the rate of emergence in the field (Appendix E3). This may be due to rubbing removing excess seed cortex material containing germination inhibitors (Santos and Pereira 1989) or reducing the layers which have to absorb water before the embryo can imbibe water. Akeson *et al.* (1980) showed that removing the pericarp from the sugar-beet fruit substantially reduced the water requirement for germination, particularly under low water conditions, so that rubbing may reduce the water requirement for a fruit to germinate. This would be particularly noticeable if seeds experienced dry soil conditions.

4.2.3.2. The performance of seedlots of cv. Rizor in the field

The RNA/DNA ratios of the ten cv. Rizor seedlots did not correlate significantly with their performance in the field (Table 3.19). When the data were ranked for each seedlot (using Spearman's correlation analysis) the rank correlation coefficients were significant for

RNA/DNA ratio 2 with field MET and T_{50}. This indicates that although RNA/DNA ratio 2 is not linearly related to emergence in the field, the RNA/DNA ratio does estimate accurately the relative field performance of seedlots at least from this cultivar. In addition there were few significant correlations between the germination test data and field performance measurements of the cv. Rizor seedlots (Table 3.20); again this may be a reflection of the similarity of the vigour of seedlots from within a single cultivar produced in one year.

4.2.3.3. The performance of three cv. Cyrano seedlots in field trials 1-3

Three seedlots from cv. Cyrano, B, G and H were sown in three field trials in order to determine the uniformity of results between trials (Appendix E5). These three trials were carried out at the same time although trial 3 took place on a different site. The seedlings in trial 3 suffered from a high level of bird damage. Trials 1 and 2 were surrounded by an electric fence and each newly-emerged seedling was marked with a cocktail stick unlike trial 3. In general, cv. Cyrano B performed the worst of the three seedlots in all three trials except for T_{50} in trial 3. Neither seedlot G nor H performed consistently better than the other in the three trials. This may be because the seedlots responded differently to conditions experienced in each trial or that seedlots G and H were very similar in terms of vigour. The first explanation is supported by the observation that seedlot G performed consistently worse than seedlot H in all of the germination tests, and had a lower RNA/DNA ratio. Alternatively, both explanations may be true because background variation may overshadow small differences in vigour levels between the lots.

4.2.4. **Field trial 4**

In this field trial, two seedlots from three cultivars were sown in order to compare inter-cultivar vigour levels assessed in the laboratory and in the field. In addition to the other measurements made in the first three trials, seedlings were harvested at establishment and then weighed immediately or following a drying period. It was also possible to divide the seedlings in to those which had emerged first (a), those which emerged in the middle of the counting period (b) and those which emerged in the later period of the counting period (c). In general, the dry weights were 10% of the fresh weights so that differences which were significant for fresh weights were also significant for dry weights.

The seedlot sown significantly affected some of the field trial measurements; these being sum%, establishment% and T_{50}* and certain seedling weight measurements. Plot location in the field was not a significant factor affecting performance, apart from the fresh weight of early emerging seedlings (a) and the dry weight per plant of those seedlings emerging in the middle of the counting period (b). However, even though plot location was not a significant factor, if the establishment% of all the seedlots in a block are averaged then the effect of position in the field trial can be seen. Two blocks, positioned in the corners of the field trial nearest to the buildings and bank, had an average establishment of 45% and 50% compared to 58-61% for the other blocks. This positional effect may have been due to one side of the trial being protected by a bank leading to a temperature gradient across the trial. An alternative explanation could be that the two blocks suffered localised grazing although the mean sum% is also low for the two blocks indicating that it is not increased post-emergence losses which caused the reduction in seedling numbers. Differences between blocks may be due to uneven soil conditions.

4.2.4.1. Relationships between the RNA/DNA ratios and field trial measurements

There were no significant correlations between the RNA/DNA ratios of the seedlots and the field performance measurements (Table 3.21). The larger, but non-significant, correlation coefficients were generated when RNA/DNA ratios 3, 7 and 8 were compared with sum% and establishment%. These correlation coefficients were also negative which was in contrast to the same comparisons made in the seedlots 1 experiment. Two correlation coefficients were significant when harvested seedling weights were compared against the RNA/DNA ratios of the seedlots (Table 3.22). However, both of these correlations between RNA/DNA 3 and total fresh weight and total dry weight were negative. This unexpected result would indicate that seedlots which produced more biomass per plot and could be said to be of higher vigour, had smaller RNA/DNA ratios. The Spearman's rank correlation analysis also produced the same negative coefficients which indicates that not only is the linear relationship negative but also that the rankings are the opposite of the expected. In general, many of the correlation coefficients between the RNA/DNA ratios and the seedling weights were negative. This contradicts the hypothesis that seeds of higher vigour have larger RNA/DNA ratios. However, because there are only six means plotted in analysis each time and if only one of those means were aberrant, then the whole relationship would be changed.

4.2.4.2. Relationships between the germination test results and field trial measurements

If the germination test results of the same six lots are compared against the field trial measurements then few of the correlations are significant (Table 3.24). One of the few significant correlations was the H% on day 4 of the wet stress test which correlated significantly with field MET and T_{50}*. This germination test measurement also accurately predicted field performance of the different seedlots in field trial 1. In general, the rates of germination in the cold stress test correlated with the rates of emergence in the field. Also, $H\%_{20C,d7}$ and $H\%_{20C,d14}$ correlated significantly ($p<0.05$) with sum% and to a lesser extent, with establishment%.

Few of the other laboratory tests ie. RNA/DNA ratios and germination tests were accurate at predicting relative field performance. Possibly, this was due to the field performance in this trial not being a true reflection of seed vigour due to an unexplained variable eg. soil capping. Durr *et al.* (1992) describe the importance of seedbed preparation and placement on early growth and seedling size.

4.2.4.3. Relationships between harvested seedling weights and field emergence measurements

It would be expected that the weight of harvested seedlings would be influenced by the number of seedlings per plot (Table 3.23). This was indeed shown by the establishment count correlating significantly with the total weight of harvested seedlings (total fresh and total dry) of each plot. When the seedlot data were ranked it was also revealed that the seedlots which had the largest establishment% also had the largest weight of seedlings which emerged in the middle (fresh and dry b) and near the end of the counting period (fresh and dry c). The sum%, which it should be noted, does not take into account seedling death once it has been counted as having emerged, also correlated significantly with the weight of seedlings which emerged late in the counting period (dry c and fresh c). Although, the weights of late emerging seedlings and total weights per plot were correlated with the sum% and establishment%, early seedling weights (fresh and dry weights a) were only correlated with rate of emergence (MET and T_{50}*). This indicates that the total weight of seedlings per plot which emerged in the earliest period was dependent on the rate of emergence of that seedlot. For example, a seedlot with a fast rate of emergence will produce more seedlings in the early period which will go on

to produce a larger seedling harvest. However, the average weight of each seedling (weight/plant a) was not significantly correlated with rate of emergence as would be expected. This lack of relationship indicates that in the early stages of crop growth, the seedlots which emerged the quickest do not necessarily produce the largest seedlings. In the later stages of crop establishment, the plot weight of the emerging seedlings is dependent on the number of seedlings present and the average weight of each seedling is correlated with field T_{30}.

4.2.4.4. Relationships between the germination test results and harvested seedling weights

It was found that the rates of germination in the cold stress test correlated with fresh and dry plot weights of seedlings which emerged early on in period a (Table 3.25). The cold stress test results also correlated significantly with rate of emergence in the field. Therefore, the rate of germination of the seedlot in the cold stress test is a good indicator of rate of emergence in the field (trials 1, 2 and 4), and also of the plot weight of seedlings which emerged early on. A seedlot which performs well in the cold stress test, will emerge more quickly in the field and produce a larger weight of seedlings.

G% and H% of the standard germination test at 20°C correlated with the plot weights of seedlings which emerged later in the counting period (dry and fresh c). Also $H\%_{20C,d7}$ and $H\%_{20C,d15}$ correlated with sum% in the field. The plot weights of late emerging seedlings (dry and fresh c) correlated with sum% in the field so, for example, a seedlot which performed well, in terms of H% at 20°C may also show greater emergence in the field (sum%) and produce a greater plot weight of seedlings which emerged later on (period c). Therefore, the measurement of H% at 20°C (day 7 and 15 only) is a good indicator of sum% in the field and later seedling plot weights.

4.2.5. **The importance of laboratory tests to estimate performance in the field**

In seedlots 1 experiment, which assessed a total of 17 seedlots from three cultivars, the RNA/DNA ratio correlated significantly with most of the germination test measurements and could therefore be used to predict field performance. Certain germination tests (T_{50} and MGT in the cold stress test and the standard germination test at 15°C, $G\%_{20C,d3}$ and $H\%_{wet,d4}$) correlated significantly with all the field trial measurements and may thus also have predictive

value. When the seedlots from each cultivar were analysed, there were no significant correlations between the RNA/DNA ratios and the field trial measurements indicating that the RNA/DNA could be used to predict the relative field performance between cultivars but not between seedlots from the same cultivar. Similarly, when individual cultivars were analysed by various germination tests there were few significant correlations with field trial performance.

Therefore it appears that in general the germination tests and RNA/DNA ratios estimate field performance at the cultivar level but are not able to differentiate consistently between the seedlots of each cultivar possibly because the seedlots used in this work were very similar. This proposition was tested in seedlots 2 experiment when two seedlots from each of three cultivars were selected. It was found that the RNA/DNA ratios did not accurately predict field performance nor the weight of seedlings which were harvested to estimate plant vigour. Indeed, few germination test measurements correlated with the field performance measurements. One measurement, $H\%_{wet,d4}$, was more consistently correlated with field performance than the other germination tests. This measurement correlated significantly with two estimates of rate of field emergence in field trial 4, with field performance measurements in trial 1 for the 17 seedlots, and well for most of the field performance measurements in trial 1 where only the cv. Cyrano seedlots were analysed. Lovato and Cagalli (1992) showed that the germination percentage on day 7 of a wet stress test and of the standard germination test at 20°C correlated with field emergence.

In addition, the cold stress test for seedlots 2 experiment correlated significantly with both the rate of field emergence (T_{30} and T_{50} *) and the plot weight of harvested seedlings which emerged early on in the counting period (weight a). In comparison, the $H\%_{20C,d7}$ and $H\%_{20C,d15}$ were significantly correlated with field sum% and the plot weight of harvested seedlings which emerged later in the counting period. This germination test in effect measures the percentage of seeds in a seedlot which can produce a seedling taller than 2 cm under optimal conditions. Therefore, results from field trial 4 indicate that some germination test measurements estimate certain aspects of relative field performance such as the number of seedlings which emerge and the weight of seedlings at establishment.

Durrant *et al.* (1985a) found that the standard germination test predicted field performance

more accurately than a range of vigour tests because although the vigour tests magnified differences between the seed samples, precision for predicting field performance was low. Bekendam (1986) found that the germination capacity of sugar-beet seeds was well correlated with field emergence and therefore the standard germination test was enough to predict growth in the field. Results from these experiments, particularly field trial 4, indicate that this was not always true, in fact certain vigour tests eg. the cold stress test were sometimes better than the standard germination test.

Basing vigour assessments on the standard germination test assumes that germination at 20°C will reflect the emergence level, rate of emergence and establishment of a seedlot under field conditions. The germination level at 20°C gives an indication of viability and therefore potential emergence and establishment levels but a high level of germination in a test does not guarantee a good field performance. This highlights the problem of predicting the performance of a seedlot in a highly variable environment in the field.

When the seedlots underwent an advancement treatment, field performance and rate of germination in the cold stress test were markedly improved in comparison with thiram-steeped seeds. This significant difference was reflected in a larger RNA/DNA ratio. This artificial improvement in vigour was large enough to be measured by the RNA/DNA ratio and the cold stress test although there was no difference between treated seedlots in the standard germination test at 20°C.

Ultimately seed vigour is determined by the performance of the seed or seedlot in the field but how accurately do individual field trials measure the relative vigour of seedlots? Field conditions are variable so that a number of trials over different years and on different sites would need to be carried out in order to accurately assess the vigour of each seedlot. Small differences in vigour between seedlots may not be accurately assessed if only one field trial is carried out, particularly if the number of replicates is low. Three field trials were carried out in Spring 1994 in order to compare the consistency of field performance measurements for three seedlots from cv. Cyrano. One seedlot performed badly in all three field trials in comparison with the other two but the remaining two were not consistent in their relative performance. This would mean that the two seedlots would be ranked inaccurately if only one of the field trial results was used. In one respect laboratory germination tests are more

appropriate than field trials because each seed is more likely to experience the same conditions, but their major problem is that only a few stresses can be imposed at one time.

In field trials, seedling measurements may be affected unequally across the trial. For example, Durrant *et al.* (1985a and b) found that early emerging seedlots were grazed more by birds in comparison with later emerging seedlings. It was found that if seedlings were protected from grazing, then several laboratory tests became more reliable predictors of field performance. In field trials 1, 2 and 4, establishment% (which included post-emergence losses) and sum% (the summation of the daily emergence counts which does not take into account post-emergence losses) were calculated. However, unlike the results by Durrant *et al.* (1985b), sum% did not correlate significantly with more laboratory tests than the establishment%.

If experimental variations could be eliminated, would field trials always rank seedlots in the same order? Obviously, seeds will respond differently to field conditions, particularly extreme ones, even within a seedlot. The seed's response to stresses will be affected by the seed's history and its genetic make-up. Seed breeders aim to improve the seeds' responses to these stresses by selecting for favourable traits such as drought stress tolerance. Therefore, the consistency of rankings will depend upon the degree of similarity of seed responses within a seedlot.

4.3. FACTORS AFFECTING THE RNA/DNA RATIO

4.3.1. **Quantification and calculation of the RNA/DNA ratio**

Different calculations were used to determine the RNA/DNA ratio because the degree of contamination of the nucleic acid samples and the most accurate method of calculation was unknown. Therefore, a whole range of calculations for determining the RNA/DNA ratio were used:

- RNA/DNA 2 was generated using the Warburg-Christian formula which includes the average absorption coefficients for nucleic acids and proteins at 260 nm and 280 nm (Müller *et al.* 1993).
- Calculation 3 took into account the absorbance of not only protein but also phenolics

which could have contaminated the sample during the nucleic acid extraction procedure or were present in the true seeds themselves. This calculation contributed to unexpected negative correlation coefficients for the seedlots 2 experiment.

- RNA/DNA 4 included a measurement to account for turbidity caused by light-absorbing particles which remained in suspension. This measurement is possible at 320 nm because nucleic acids and proteins do not absorb at this wavelength.
- Due to possible differences in the measurements of DNA concentration by the spectrophotometer and fluorimeter, a calibration curve was generated using DNA standards which were measured on both machines. During the calculation of RNA, the equivalent absorbance of DNA, which had already been quantified using the fluorimeter, could then be interpolated from the calibration curve. The same measurements of contamination used in RNA/DNA 3 and RNA/DNA 4 were also included in calculations 7, 8, 9 and 10. RNA/DNA 6 differed from RNA/DNA 5 only in the inclusion of absorption coefficients which did not affect the correlation coefficients generated when the RNA/DNA ratios were compared with the other assessments of vigour. The correlation coefficients for RNA/DNA 7 with 8 and RNA/DNA 9 with 10 were recorded together in the results tables for the same reason. RNA/DNA 7 and 8 produced larger (but still non-significant) correlation coefficients than the other calculations when they were compared with the germination test measurements for seedlots 2 experiment. However, when the RNA/DNA ratios were compared with the field trial measurements, unexpected negative correlation coefficients were seen between RNA/DNA 7 and 8 with sum% and establishment%.

All the RNA/DNA ratio calculations were included in the analyses for this thesis because different calculations correlated significantly with each germination test and field trial measurement. Overall, most of the RNA/DNA ratio calculations generated a similar number of significant correlation coefficients except for RNA/DNA ratios 3, 7 and 8 which tended to produce unexpected relationships when they were compared with other methods of vigour assessment. If the vigour test was to be used commercially then ideally only one calculation would be used. RNA/DNA ratios 1 and 5 are the simplest calculations and are no worse than the other methods. Therefore, either calculation would be suitable.

4.3.2. **Fruit size**

In order to investigate the correlation between fruit size and the RNA/DNA ratio, no dead seeds or true seeds from clusters or polyembryonic fruits were included for analysis. The RNA/DNA ratios increased with fruit size although fruit-size grades 9 and 10 had smaller RNA/DNA ratio values than would be expected (Appendix D4). Akeson *et al.* (1981) found that for sugar-beet seeds, emergence increased with fruit size except for the largest size. This might be explained by the fruits of grades 9 and 10 possessing more seed cortex material and not larger true seeds (Sarah Yallop-personal communication). In the field, the larger fruits germinate more quickly (MET and T_{50}) and produce bigger seedlings (dry weight/plant).

The amount of extractable DNA and extractable RNA increases with fruit size except for size grade 10. Also, the relative amount of extractable RNA to DNA increases as fruit size increases which generates the increasing RNA/DNA ratio (except grades 9 and 10).

This increase in the RNA/DNA ratio with fruit size may be due to the true seeds being more mature and more vigorous. Seeds which were more mature at harvest have been shown to be more vigorous in carrot (Brocklehurst and Dearman 1980) and wheat (Dell'Aquila and Tritto 1991). More mature carrot and *Nicotiana spp.* seeds have been shown to contain more intact rRNA than immature seeds (Brocklehurst and Fraser 1980).

Although there is a significant difference between the RNA/DNA ratios of fruit-size grades studied in this work, the size range of commercial seeds is much smaller (fruit-size grades 1 to 4). Therefore, using the RNA/DNA for testing seed vigour of commercial seedlots should not be affected by fruit size distribution within the seedlot as indicated by trends seen for cv. Zulu.

The results were generated using a cultivar which had previously shown that larger fruit fractions performed better in the field (Thomas and Yallop 1994). Recently, studies using size-graded material from additional cultivars have not shown such clear trends in the field (Sarah Yallop-personal communication). Therefore, it would be useful to determine the RNA/DNA ratios of these size fractions to investigate whether larger RNA/DNA ratios are linked with improved field performance and not just larger fruit size.

4.3.3. **Ploidy**

A triploid cell possesses three sets of chromosomes whereas a diploid cell has two sets. Therefore, triploid cells might possess more copies of DNA and therefore have more extractable DNA and RNA relative to the cells from diploid bulks. It might be expected that triploid cultivars have in general a larger RNA/DNA ratio and to test this hypothesis cultivars of similar germination performance were selected. The amount of extractable DNA was larger in the triploid of every pair in comparison with its diploid of the same germination performance (Appendix D7). This was also the case for the amount of extractable RNA. There was no significant difference between the RNA/DNA ratio of diploid and triploid lots which had similar germination test results. Indeed, an analysis of variance of the results shows that ploidy is not a significant factor affecting the RNA/DNA ratio. Therefore ploidy does affect the levels of extractable nucleic acids but not the RNA/DNA ratio, based on the limited number of comparisons made here.

4.3.4. **Seed treatments**

There was no significant difference between the RNA/DNA ratios of thiram-steeped seeds and untreated seeds although the thiram-steeped seeds had slightly smaller RNA/DNA ratios (Appendix D8). In comparison with untreated seeds, the steeped seeds showed an increase of 7-15.5% in extractable DNA indicating that the steeping period of six hours may have been long enough to facilitate a small amount of DNA synthesis or DNA repair. DNA repair would reduce the fragmentation of the DNA to allow more higher molecular weight DNA to be extracted. The amount of extractable RNA following the steeping process usually decreased, possibly due to RNA degradation, but increased slightly for one seedlot.

A significant increase in the RNA/DNA ratio in the advanced seeds in comparison with thiram-steeped or untreated seeds was shown in seed treatment experiments 1 and 2 (Appendix D8 and D10). The advancement treatment increased the level of extractable RNA (RNA/DNA calculation 1) by 50-100% of the thiram-steeped seed level and the extractable DNA by 20-35% depending on the cv. Cyrano seedlot analysed. Work by Coolbear and colleagues (Coolbear and Grierson 1979, Coolbear *et al.* 1980) demonstrated that there was a large increase in RNA relative to DNA during priming of tomato (*Lycopersicon sp.*). Bray *et al.*

(1992) suggests that priming allows for the replacement of damaged molecular components such as rRNA. This synthesis of larger rRNA species during priming was demonstrated in leek embryos using polyacrylamide gel electrophoresis. DNA synthesis during seed treatments will be discussed in more detail in section 4.4.1.

The RNA/DNA ratios of the aged seeds did not reflect the loss in germination seen in the standard germination test at 20°C. Ageing did not increase the number of empty or abnormal true seeds when the fruits were dissected. The result is in contrast to a decrease in the RNA/DNA ratio seen in leek seeds following accelerated ageing (Clarke and James 1991). These different observations may be explained by damage being dependent on the type of ageing treatment. In maize, for example, two types of ageing process decreased the recovery of DNA polymerase to different extents (Vázquez-Ramos *et al.* 1988, Vázquez *et al.* 1991). In addition, the decrease in germination may be due to the accelerated ageing process damaging another part of the cell eg. cellular membranes and not the nucleic acids. Also, it has been found that ageing increased the fragmentation of DNA in rye and *Secale cereale* embryos but did not affect the total DNA levels (Hallam *et al.* 1973, Osborne *et al.* 1980/81). Indeed, the slight increase in RNA following ageing may be due to the increased moisture content facilitating RNA synthesis because the artificial ageing treatment has been described as an extreme form of priming (Priestley 1986) and priming has been shown to increase the RNA content (Khan *et al.* 1978, Davison *et al.* 1991). An increase in metabolic activity has been seen following six days of an ageing treatment of sorghum seeds where certain enzymes, eg. amylase, increased in activity (Perl *et al.* 1978).

There was an increase in abnormal seedlings after artificial ageing, especially if the seed had first been advanced, where 10% of seeds produced abnormal seedlings. This may be due to the advancement treatment making the seeds more susceptible to deterioration, as has been seen in pepper seeds following a priming process (Saraceo *et al.* 1995). Advanced sugar-beet seeds deteriorated over three years of storage so that their field performance was the same as thiram-steeped seeds (Thomas *et al.* 1993).

4.4. DNA SYNTHESIS IN RELATION TO SEED TREATMENTS AND VIGOUR

4.4.1. **Seed treatments**

The cell cycle is conventionally described in terms of four phases: G_1 (main growth phase), S (DNA synthesis), G_2 and M (mitosis). The 1C value corresponds to the DNA content of the unreplicated haploid chromosome complement (Bennett and Smith 1976). At the completion of maturation in most plant embryos, the cycle arrests in the G_1 phase with a 2C DNA content (Baker and Bradford 1995). Accordingly in this work, the nuclei in the untreated sugar-beet embryo root tip and in the leaf tissue were predominantly 2C (Table 3.28).

When the H.M.W. DNA extracted from cv. Cyrano G seedlot was quantified as part of determining the RNA/DNA ratio, the amount of DNA, extracted from 50 true seeds, increased by 36% following the advancement treatment (Appendix D9). This increase in extracted DNA could be due to an increase in the number of cells or to the DNA content of some cells doubling. An increase in 4C nuclei would indicate that the individual cellular DNA contents had increased during the advancement treatment.

The percentage of root tip nuclei with a DNA content of 4C or larger than 4C was calculated for untreated seeds, advanced seeds, artificially aged seeds and advanced then aged seeds from one cv. Cyrano seedlot (Table 3.28). Advanced seeds showed a higher proportion of nuclei with DNA contents larger than 2C in comparison with untreated seeds; the percentage of nuclei with a 4C DNA content rose from 2% to 14% with 10% of additional nuclei having a DNA content greater than 4C. This indicates that the advancement treatment facilitates DNA replication but cells are arrested at the post S-phase because no cell division was seen in the advanced root tip cells. These results are similar to those determined using flow cytometry in pepper (Lanteri *et al.*1993, 1994) and tomato root tip cells where osmopriming increased the percentage of 4C nuclei (Bino *et al.* 1992, Lanteri *et al.* 1994, Baker and Bradford 1995). The absence of cell division occurring in the advanced root tip cells of sugar beet was also seen in leek seeds during priming where DNA synthesis occurred without cell division (Bray *et al.* 1989). The presence of detectable DNA replication and cell division during a seed treatment is dependent on the length and conditions of the priming treatment: for example, no DNA replication or cell division was seen during the osmopriming of maize seeds (Cruz-Garcia *et*

al. 1995).

There is good evidence shown in this thesis that the acceleration in rate of germination by advancement or priming treatments facilitates DNA replication without cell division. The improvement in field performance and in the cold stress test by advancement, such as that seen in the present work, may be explained by the seeds containing nuclei which are ready to divide as soon as they come into contact with water. Indeed, Lanteri *et al.* (1994) found that the advancement of germination in the laboratory (the reduction in MGT), following the osmopriming of tomato and pepper seeds, was significantly ($p<0.01$) correlated with the percentage of 4C nuclei. However, Saracco *et al.* (1995) found that a priming treatment which increases pepper seed performance did not generate an increase in the 4C content.

Artificial ageing decreased the percentage of 4C root tip nuclei. In the advanced seeds, for example, ageing decreased the percentage of nuclei with 4C DNA contents from 14% to 5%. This indicates that a small amount of cell division (4C to 2C DNA contents) occurred during the artificial ageing treatment. Advanced or primed seeds are generally known to be more susceptible to deterioration during storage (Baker and Bradford 1995). A seed treatment which improves seed performance but does not facilitate DNA replication might therefore perhaps produce seeds with better storage characteristics.

For sugar-beet seeds, an increase in seedling abnormalities was seen in the advanced seeds which had been artificially aged. In normal aged seeds, the percentage of seedling abnormalities was c.a. 1.4% (although the normal aged seeds had a lower germination level) whereas in advanced then aged seeds it was nearly 10%. This demonstrates that advanced seeds are more sensitive to artificial ageing.

If a priming or advancement process is extended for too long a time then seeds are damaged when they are dried back to the original moisture content (Davison *et al.* 1991). This may be due to the nuclei passing too far through the cell cycle and becoming desiccation sensitive. It has been shown that during imbibition, seeds become desiccation sensitive if they are dried back after a critical time (Deltour and Jacqmard 1974, Sen and Osborne 1974, Crèvecoeur *et al.* 1988). Irreversible damage to chromatin and nuclear membranes occurs if dehydration occurs in the 36th hour (or later) of germination in *Zea mays* and *Sinapis alba* seeds (Deltour

1985). In these experiments reported here, the drying back stage following the advancement treatment evidently did not reduce seed performance so that the post S-phase, at which the sugar-beet embryos arrested, is not desiccation sensitive. In maize, drought resistance is lost when the radicle cells enter the S-phase (Deltour and Jacqmard 1974) whereas Bernie and Drennan (1971) place loss of desiccation tolerance at a later time ie. at the start of cell division. It therefore appears that the latter may also be the case for sugar-beet embryos.

4.4.2. **Vigour**

Three cv. Cyrano seedlots were selected for their performance in the laboratory germination tests. This enabled the seedlots to be ranked approximately in terms of decreasing vigour in the order: H, B then G. It has been shown that cell division is arrested once the moisture content drops below a critical level during seed maturation (Osborne 1981). The final proportion of G_1 and G_2 cells in the tissues of dry embryos depends on the time between the complete inhibition of DNA synthesis and that of mitosis (Deltour 1985). Sliwinska (1995) has shown that a lower vigour cultivar has a higher G_2/G_1 ratio. The observation that seeds with a lower 4C% are more vigorous may be explained by the fact that cells containing 2C nuclei are less sensitive to damage (rev. Deltour 1985). Seeds with an increased 4C% due to a priming treatment are more sensitive to controlled deterioration (Saracco *et al.* 1995). By contrast, in the present experiments on untreated seeds, it was found that the more vigorous seedlot, H, had the highest percentage of 4C nuclei in comparison with lots, B and G. However, these changes were small and measurements were made on fewer nuclei using Feulgen staining and image analysis instead of flow cytometry. Also the work by Sliwinska used three cultivars selected for high, medium and low vigour whereas the experiments here compared intra-cultivar differences in vigour.

The proportion of nuclei with 4C DNA contents in an embryo root tip appears therefore to be due to the interplay of different factors. It has been shown here that the primed advancement treatment increases the percentage of 4C nuclei and that this treatment is associated with improved seed performance. Unfavourable storage conditions and seed maturity may decrease the percentage of 4C nuclei. The level of 4C may not directly affect seed performance but rather be a result of and therefore be indicative of the seed's history.

4.5.1. **Preliminary experiment**

When DNA samples are run on an alkaline gel the DNA duplex is dissociated in to single strands. The higher molecular weight DNA strands are found closest to the origin and the molecular weight decreases progressively down the gel. The fragmentation profile of the control embryos had the highest intensity reading at approximately 20 units from the origin whereas the γ-irradiated embryos had a DNA profile which peaked at approximately 60 units. Irradiation of the embryos increases the fragmentation of the DNA by introducing additional single strand breaks which is seen as an increase in smaller molecular weight DNA compared to unirradiated embryos (Tano and Yamaguchi 1977, Elder and Osborne 1993). When the irradiated embryos were imbibed for two hours, the DNA profile moved to a higher molecular weight, with the maximum intensity at around 20 units from the origin.

The amount of DNA in each section down the gel was measured so that segment A refers to the measurements nearest to the origin which corresponds to the highest molecular weight DNA fragments. The smaller DNA fragments migrated further down the gel so that segment D included the smallest molecular weight fragments. When the percentage of the total intensity readings for each segment was calculated (Table 3.30), it was found that the DNA from the control embryos had nearly 20% of the total fragments in the highest molecular weight segment A whereas the irradiated embryos had just over 10%. Following an imbibition period of two hours, the percentage of the total fragments in the highest molecular weight segment increased to 19%. The imbibition period at an optimal temperature facilitates a ligation of the DNA fragments to produce more higher molecular weight DNA. This has also been reported by Tano and Yamaguchi (1977) and Elder *et al.* (1987) who have indicated that this process could be described as DNA repair.

This first experiment demonstrated that differences in the fragmentation of DNA extracted from γ-irradiated and irradiated-imbibed sugar-beet embryos could be detected so that two seedlots of different vigour were selected in order to assess any differences in DNA repair capacity.

4.5.2. **The effect of vigour on DNA repair at an optimal temperature**

There were no consistent differences found between the extent of DNA repair of high and low vigour embryos, during imbibition following the irradiation treatment (Table 3.31 and Table 3.32). The seeds used were commercially available and therefore of high quality so that the differences between high and low vigour were small. In this experiment, imbibition, which facilitates DNA repair, took place at an optimal temperature of 24°C. Earlier work has shown that there are smaller differences between the performance of the high and low vigour seeds when they are germinated at 20°C than when they are germinated under stressful conditions such as at 9°C. Similarly, there were larger differences between high and low vigour wheat embryos in terms of levels of poly $(A)^+$-RNA (Smith and Bray 1982), rates of protein and RNA synthesis (Blowers *et al.* 1980) and levels of nucleotides (Standard *et al.* 1983) during imbibition at 10°C rather than at 20°C.

The extent of DNA repair, under stressful conditions, of artificially damaged DNA may well be greater for the high vigour seeds compared to the low vigour seeds. Therefore two seedlots were selected for their different germination performance at 9°C but similar response at 20°C to test this hypothesis (section 4.5.3.).

Although only a limited number of embryos were tested, it was found that the irradiated embryos did not germinate as well as the controls. The irradiated and irradiated-imbibed embryos showed a similar germination level but the health ranking of the irradiated-imbibed embryos was lower than the irradiated embryos (Table 3.31). This is unexpected because irradiation causes abnormalities in seedlings and the two hour imbibition period, which allows DNA repair, should have improved the quality of the DNA and therefore decreased abnormalities in the seedlings. Other problems in the embryos may have been produced following the irradiation treatment which may not have been improved by a two hour imbibition period. For example, Berjak and Villiers (1972) showed that there was a delay in the germination of aged *Zea mays* caryopses in order to allow the repair of organelles. The decrease in the health of the seedling could be explained by the cells suffering overall damage even though DNA repair was still functioning. This is supported by the fact that *Avena fatua* embryos, which are in a state of dormancy can still repair DNA which has been fragmented by γ-irradiation (Elder and Osborne 1993). Alternatively, the imbibition stage, followed by

drying back before the germination test, may have allowed infection by micro-organisms or, the increase in handling may have damaged the delicate embryos. For the irradiated-imbibed embryos, the germination test was the third occasion of imbibition so that seed food reserves or, for example, nucleotide pools may have become depleted. Advancing treatments, which may involve frequent wetting and drying cycles, improve vigour but differ from this present case in using the whole true fruit so that the embryo is protected. In commercial seed treatments, the wetting and drying cycles are optimised to prevent damage to the embryo.

4.5.3. **The effect of vigour on DNA repair at a sub-optimal temperature**

The previous experiments demonstrated that the irradiation treatment created greater DNA fragmentation in comparison with the control. In this experiment, the fragmentation profiles of the controls were inconsistent between the replicates and even for the same samples run on different gels. This lack of consistency could not be attributed to an obvious variable. Therefore, only the DNA fragmentation profiles of the irradiated and irradiated-imbibed embryos were compared.

The high vigour seedlot, in general, showed a greater improvement in terms of the recovery of higher molecular weight DNA during imbibition following the irradiation treatment. This indicates that the higher vigour lot repairs DNA better than the lower vigour seedlot at the lower temperature. This vigour difference was also seen at a lower temperature for rate of RNA and protein synthesis (Blowers *et al.* 1980). One DNA sample (cv. Cyrano F, replicate 2) run overnight on gel 1 (Table 3.34), showed only a small increase in the percentage improvement in higher molecular weight DNA even though it was from the high vigour seedlot. This sample when run again on gel 2 overnight or run for five hours (Table 3.35), showed a much larger percentage recovery of higher molecular weight DNA. Therefore these experiments need to be repeated in order to verify this larger DNA repair capability in the embryos with higher vigour.

The ability to repair DNA in the first few hours may be essential to fast germination. During storage, DNA becomes fragmented (Osborne *et al.* 1980/81) and therefore before DNA can be synthesised, the template needs to be as intact as possible. In addition, DNA needs to have been repaired before the cell will be allowed through checkpoints in the cell cycle. This has

been seen in mammalian cells treated with DNA damaging agents where cells become delayed at checkpoints in G_1, S and G_2 phases (O'Connor *et al.* 1993). Cell division has been shown not to be essential to germination (Bewley and Black 1978) but cell division will become important as the seedling develops. Damaged DNA has been shown to delay germination; low viability and vigour seeds which germinate more slowly, possess more fragmented DNA (Sen and Osborne 1974). Also, lower vigour seeds show a greater deterioration in laboratory performance, DNA synthesis and DNA polymerase activity following ageing (natural and artificial) and take longer to recover in contrast to higher vigour seeds (Guitierrez *et al.* 1993). Therefore, a DNA repair system which can operate quickly and efficiently during the first hours of imbibition will affect rate of germination and thereby emergence, both processes being important characteristics of high vigour.

4.6. FUTURE WORK

The absence of significant correlations between any of the laboratory tests, including the RNA/DNA ratio, and the field trial measurements for the second experiment of assessing seedlot vigour highlights the difficulties of assessing seed vigour in the field and in the laboratory. Firstly, there is a need to repeat field trials in different years and on different sites using the same seedlots, even though field trials require a large input of resources. Secondly, it would be interesting to determine the RNA/DNA ratios for very low quality seed material which has not been artificially aged because this series of experiments used high quality commercially-available seeds. Using seedlots with more extreme vigour characteristics but similar viability would be useful to determine a clearer picture of DNA repair and DNA contents in relation to vigour. Also it would be interesting to measure the RNA/DNA ratios of seeds at different stages in the primed advancement treatment rather than on completion of the treatment.

The experiments carried out so far do not rule out the possibility of using the RNA/DNA ratio to estimate seed quality. There is also a practical need for the test to be simplified by, for example, reducing the number of steps for the extraction of the nucleic acids. Commercially, the RNA/DNA ratio could be used as a method for checking that the primed advancement treatment has been carried out.

The importance of seed vigour as part of seed quality will continue to be of interest. As molecular biological techniques improve, there are possibilities of investigating the control of vigour at the gene level and also finding markers linked to high vigour. Fujikara and Karssen (1995) have already partially sequenced a protein which is expressed more in osmoprimed cauliflower seeds than in control seeds and less in artificially aged seeds. A potential obstacle to this type of study is that vigour encompasses a wide range of characteristics unlike, for example, resistance to a specific disease and therefore vigour is more likely to be under the control of many genes.

Clarke and James (1991) demonstrated that the ratio of extractable RNA to extractable DNA increased following a priming treatment in leek seeds and decreased following an artificial ageing treatment. I have investigated the use of this ratio in determining vigour in sugar-beet seedlots with special reference to the benefits of the primed advancement treatment given to the seed. RNA/DNA ratios were determined for sugar-beet seedlots and compared against other methods of seed assessment (field performance and germination tests). When 17 seedlots from three cultivars (seedlots 1 experiment) were assessed and analysed it was found that there were significant correlations between the RNA/DNA ratios and the germination test and field trial measurements. This indicated that the RNA/DNA ratio was reliably predicting relative seedlot vigour in germination tests and in the field. However, when the seedlot data from individual cultivars were analysed, there were few significant correlations between the field trial measurements and the laboratory methods of seed assessment (RNA/DNA ratios and germination tests). This would suggest that the laboratory tests could estimate field performance at the cultivar level but were not able to differentiate consistently between seedlots of the same cultivar.

A second experiment (seedlots 2 experiment) was set up using two seedlots from three cultivars which were assessed again by the RNA/DNA ratio, a range of germination tests and in a modified field trial which had more replicates. In this experiment, the seedlings were harvested at establishment. Here too, there were no significant correlations between the RNA/DNA ratio and the germination test and field trial measurements (including the harvested seedling weights). Also few germination test measurements correlated significantly with field performance, for example, the wet stress test in which the percentage of hypocotyls being larger than 2 cm (H%) on day 4 correlated significantly with rates of field emergence. This germination test measurement also correlated significantly with all the field trial measurements in field trial 1 (seedlots 1 experiment). Most of the germination test measurements as well as the RNA/DNA ratio did not correlate significantly with field performance, which may be indicative of the problems of basing seed vigour assessments on one field trial at one site. For example, when three cv. Cyrano seedlots were sown in three different trials in one year, one seedlot was consistently the worst but the other two seedlots varied in their relative field performances. This could be due to the seedlots being very similar, since all seedlots used in

these experiments were commercially available and therefore of a high quality.

Although there were no significant correlations between the RNA/DNA ratio and field and laboratory performance in seedlots 2 experiment, the results do not rule out the possibility of using the RNA/DNA ratio to estimate seed quality. One problem with determining the RNA/DNA ratio is the variability in results when different calculations are compared. No particular calculation was consistently better at determining the RNA/DNA ratio. RNA/DNA ratio 3, which included a measurement of protein and phenolic contamination, was the least successful calculation in correlating significantly with the germination test and field trial measurements. These results also highlight the difficulties of assessing seed vigour in the laboratory because vigour encompasses a wide range of characteristics whereas each germination test may only be measuring the response of a seedlot to one set of conditions. In the field, small differences in vigour between seedlots may be masked by background variation. By comparing seedlots which had undergone a primed advancement treatment with thiram-steeped seedlots, larger differences in seed performance could be investigated.

An artificial increase in seed vigour produced by the primed advancement treatment was reflected as an increased RNA/DNA ratio. Advanced seeds showed significantly improved field performance, faster germination in the laboratory test at 9°C and a significantly higher RNA/DNA ratio in comparison with thiram-steeped seeds. The RNA/DNA ratio could therefore be used commercially to ensure that the advancement treatment has been carried out. The advancement treatment increased the amount of extractable RNA by 50-100% and extractable DNA by 20-35% compared to the thiram steep treatment.

The effect of seed treatments such as thiram-steeping and artificial ageing on nucleic acid levels was also studied. There was no significant difference between the RNA/DNA ratios of thiram-steeped seeds and untreated seeds although the amount of extractable DNA increased by 7-15.5% following the thiram-steep. The effect of the artificial ageing treatment on the RNA/DNA ratio did not reflect the decrease in germination performance seen in the standard germination test at 20°C, unlike the decrease in the RNA/DNA ratio seen in leek seeds by Clarke and James (1991). Seeds which were aged after an advancement treatment produced seedlings of which 10% were abnormal when germinated in standard conditions. Advanced or primed seeds may be more susceptible to deterioration during storage (Baker and Bradford

1995) which may explain the increased number of abnormal seedlings when the advanced-aged seeds were germinated.

The effect of fruit size and ploidy on the RNA/DNA ratio were also investigated. True fruits from one cultivar were graded by size (grades ranged from less than 3.5 mm in diameter to larger than 5.5 mm in diameter). It was found that, in general, the amount of extractable RNA increased relative to that of DNA as fruit size increased. This means that the RNA/DNA ratio was higher for the larger fruits which may be due to them being more mature. Carrot and wheat seeds which were more mature at harvest have been shown to be more vigorous (Brocklehurst and Dearman 1980, Dell'Aquila and Tritto 1991). This is supported by results from field trial 3 because the higher density seeds showed higher establishment and emerged more quickly than medium density seeds. There was no difference between the RNA/DNA ratios within the commercial seed size range of 3.5 mm to 4.25 mm in diameter.

In order to investigate the effect of ploidy on the RNA/DNA ratio, triploid and diploid cultivars were selected based on having similar germination performances in the laboratory. It was found that ploidy does affect the level of extractable nucleic acids, ie. more RNA and DNA were extracted from the triploid cultivars than the diploids, but there was no clear trend in terms of the effect of ploidy on the RNA/DNA ratio.

The DNA status of root tip nuclei was examined using Feulgen staining and image analysis. It was calculated that in sugar beet there was 1-2 pg of DNA per 2C nucleus, which is of a similar magnitude to the calculation by Bennett and Smith (1976). Sugar-beet leaf nuclei were predominantly 2C as were the root tip nuclei from untreated embryos. Following the advancement treatment, the percentage of root tip nuclei with DNA contents of 4C and above rose from 2% to 24% indicating that DNA replication is occurring during the advancement treatment. Cell division was not detected in the root tip nuclei, however. Therefore the increase in extractable DNA during the advancement treatment is due to individual cellular DNA contents increasing and not an increase in the number of cells. Similarly, an increase in cellular DNA contents during osmopriming has been demonstrated in pepper and tomato root tip cells (Bino *et al.* 1992, Lanteri *et al.* 1993, 1994, Baker and Bradford 1995).

It is well known that imbibing seeds become desiccation sensitive if they are dried back after

a critical length of time. The drying back stage of the advancement treatment evidently did not reduce seed performance (demonstrated in field trial 2) so that the post S-phase at which the sugar-beet embryos are arrested is not desiccation sensitive. When advanced seeds were artificially aged, the percentage of 4C, or larger than 4C, DNA contents in the root tip nuclei decreased from 24% to 16%. This may indicate that a small amount of cell division was occurring during the artificial ageing treatment.

The effect of vigour on DNA synthesis was also studied. Three seedlots from the same cultivar were compared and it was found that the more vigorous lot had a higher percentage of 4C nuclei than the two lots with lower vigour. This contradicts work by Sliwinska (1995) who found that it was the lower vigour seedlot which had a higher G_2/G_1 ratio ie. had more 4C nuclei. The difference in results may be due to Sliwinska (1995) studying inter-cultivar differences.

An investigation in to DNA repair demonstrated that the γ-irradiation of embryos increased DNA strand breakage and decreased the amount of higher molecular weight DNA when the extracted DNA was fractionated on alkaline gels. If, following irradiation, the embryos were imbibed for two hours at 24°C, then there was a return to higher molecular weight DNA. It has been shown that during the imbibition period, a ligation of the DNA fragments can occur to produce more higher molecular weight DNA by a process described as dark excision repair (Elder *et al.* 1987). The effect of vigour on the capacity to repair damaged DNA during imbibition at 24°C was tested using two cv. Rizor seedlots. No consistent differences were seen between the seedlots in terms of the restoration of higher molecular weight DNA during imbibition. Two seedlots were then selected, based on their different germination performances at 9°C but similar response at 20°C, in order to investigate whether the effect of vigour on DNA repair capacity would be more readily expressed at the stressful low temperature. The seedlot with the higher vigour at 9°C showed a higher degree of repair of irradiation-induced DNA fragmentation, in terms of the recovery of higher molecular weight DNA during imbibition, than the lower vigour lot. The experiments need to be repeated with more seedlots to show whether vigour differences are consistently reflected in the ability to repair DNA under stressful conditions. The ability to repair DNA quickly and efficiently during the first hours of imbibition, particularly following a period of storage, would appear to exert a control on the rate of germination; an important characteristic of seed vigour.

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APPENDIX A1 The plot layout of field trial 1 (numbers 1-10=cv. Cyrano seedlots A-J; 11-13=cv. Marathon seedlots A-C; 14-17=cv. Matador seedlots A-D).

↓
N

1	2	3	4	5	6	7	8	9	10	11	12
4	8	6	13	11	10	1	5	11	13	10	16
13	14	15	16	17	14	19	20	21	22	23	24
9	5	1	16	12	14	9	17	12	3	14	2
25	26	27	28	29			30	31	32	33	34
7	2	15	3	17			6	15	8	7	4
35	36	37	38	39			40	41	42	43	44
4	13	10	6	8			16	2	12	1	6
45	46	47	48	49	50	51	52	53	54	55	56
1	15	12	2	14	17	3	5	9	8	17	10
57	58	59	60	61	62	63	64	65	66	67	68
11	9	16	7	5	3	15	14	4	7	13	11

Plot size: 1/566 hectare (12 m x 3 rows)

APPENDIX A2 The plot layout of field trial 2 (odd numbers=steeped seeds and even numbers=advanced seeds; numbers 1 and 2=cv. Cyrano seedlot B; 3 and 4=cv. Cyrano seedlot G; 5 and 6= cv. Cyrano seedlot H; 7 and 8=cv. Matador seedlot B; 9 and 10=cv. Matador seedlot C; 11 and 12=cv. Matador seedlot D).

↓
N

1 12	2 10	3 2	4 8	5 11	6 1	7 5	8 9
9 7	10 6	11 1	12 5	13 8	14 3	15 10	16 2
17 3	18 11	19 9	20 4	21 7	22 6	23 4	24 12
25 1	26 12	27 3	28 9	29 2	30 4	31 12	32 8
33 6	34 5	35 11	36 10	37 1	38 10	39 11	40 9
41 2	42 8	43 7	44 4	45 5	46 3	47 7	48 6

Plot size: 1/566 hectare (12 m x 3 rows)

APPENDIX A3 The plot layout of field trial 4 (numbers 1=cv. Cyrano seedlot H; 2=cv. Cyrano seedlot G; 3=cv. Rizor seedlot B; 4=cv. Rizor seedlot F; 5=cv. Matador seedlots B; 6=cv. Matador seedlot D).

N

1 3	2 6	3 2	4 4	5 1	6 5	7 6	8 2	9 1	10 3	11 4	12 5
13 5	14 1	15 2	16 4	17 3	18 6	19 1	20 6	21 3	22 2	23 5	24 4
25 5	26 4	27 2	28 1	29 6	30 3	31 1	32 2	33 4	34 6	35 5	36 3
37 2	38 3	39 1	40 4	41 6	42 5	43 4	44 3	45 6	46 2	47 5	48 1

Plot size: 1/4938 hectare (4.5 m x 3 rows)

APPENDIX B Statistical analyses.

Standard error of the mean

The standard error (S.E.) of the mean was calculated as follows:

$$\frac{S.D}{\sqrt{n}} \qquad \text{where the } S.D^2 = \frac{\Sigma x^2 - ((\Sigma x)^2 / n)}{n - 1}$$

(n = number of replicates of x)

Analysis of variance (ANOVA)

ANOVAs were calculated using the computer package, Genstat ™5 Release 3 (Genstat 5 Committee 1993). The assumptions of the normality of residuals and homogeneity of variances were checked for each analysis. Count data were transformed using the angular transformation:

$$x = (180 / \pi) \times \arcsin (\sqrt{} (\% P / 100))$$

where % P is a percentage with $0 < \% P < 100$.

Least significant difference (L.S.D.)

The L.S.D.s were calculated from the standard error of the differences (S.E.D.) calculated from the ANOVA tables:

$$S.E.D. \times t_{(0.05,\ resid\ df)} = L.S.D.$$

where $t_{(0.05,\ resid\ df)}$ is the tabulated t-value calculated for the residual degrees of freedom at 5% probability. The t-table used was from Snedecor and Cochran (1956).

Correlation coefficient

The Pearson's correlation coefficient, r, is calculated to determine the significance of a linear relationship. The analysis was carried out using QUATTRO PRO. The significance of r is determined by comparing r with the tabulated value for (n-2) degrees of freedom. If the observed r is larger than the absolute value of the tabulated r at 5% probability then the correlation is significant ($p<0.05$). The table of correlation coefficents used was from Bailey (1966). The significant probabilities used were $p< 0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Spearman's rank correlation coefficient

The Spearman's rank correlation coefficient, R, is a measure of association between the rankings of two variables measured for N individuals. Each individual is ranked for each variable and the Spearman's coefficient calculated:

$$R = 1 - \frac{6\sum d^2}{n(n^2-1)}$$

where d is the difference between the corresponding ranks.

The analysis was carried out using Genstat ™5 Release 3 and tests the null hypothesis of independence between the samples (Siegel 1956). If $N \geq 8$ then the student's t approximation for (N-2) degrees of freedom is calculated and if $N<8$ then significance levels from stored tables are generated by Genstat. If R is significant ($p<0.05$) then the null hypothesis, that there is no association between the samples, is rejected. The recipricol of the rate measurements (T_{30}, T_{50}, T_{50}* and MET) were calculated in order that the best seedlots, which germinated more quickly, had the largest values and could be ranked in the same way as the other seed vigour assessments.

APPENDIX C1 The germination test results of seedlots 1 experiment (mean of three replicates).

Lot	cold stress test					
	T_{50} (hours)		MGT (hours)		MHT (hours)	
	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano A	190.000	5.000	215.057	6.822	456.168	21.070
Cyrano B	207.500	0.000	214.748	2.972	478.317	12.862
Cyrano C	188.333	8.819	205.892	6.684	494.163	14.794
Cyrano D	193.333	6.667	202.206	8.604	431.855	21.240
Cyrano E	206.667	3.333	216.697	5.174	470.398	9.874
Cyrano F	191.667	4.167	216.869	9.172	440.374	10.418
Cyrano G	215.000	0.000	227.700	2.985	489.958	21.785
Cyrano H	175.000	0.000	186.660	5.367	440.251	5.532
Cyrano I	218.333	16.415	221.647	3.324	478.566	9.676
Cyrano J	190.000	0.000	199.077	1.535	449.431	1.055
Marathon A	171.667	4.410	190.059	4.529	432.017	9.322
Marathon B	168.333	3.333	195.657	4.516	486.653	27.999
Marathon C	148.333	3.333	165.386	1.736	437.892	4.432
Matador A	158.333	1.667	172.006	2.698	480.141	7.006
Matador B	145.000	5.000	169.987	2.764	445.845	14.589
Matador C	170.000	5.000	187.218	5.433	444.355	3.556
Matador D	175.000	5.000	199.275	3.061	441.397	2.092
Rizor A	161.667	12.019	175.483	13.972	370.300	34.888
Rizor B	133.333	17.638	149.167	16.213	328.800	42.081
Rizor C	131.667	7.265	144.733	7.592	347.100	15.509
Rizor D	156.667	24.037	175.967	21.584	407.033	36.096
Rizor E	178.333	4.410	193.800	6.769	399.733	8.102
Rizor F	178.333	19.221	193.700	16.089	400.067	23.133
Rizor G	146.667	17.638	132.033	15.053	329.733	39.896
Rizor H	146.667	15.899	158.600	13.554	384.967	27.633
Rizor I	156.667	8.819	167.667	4.930	383.900	19.825
Rizor J	156.667	23.333	170.067	23.114	388.667	37.948

Lot	standard test at 15°C					
	T_{50} (hours)		MGT (hours)		MHT (hours)	
	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano A	98.000	4.000	109.707	3.935	224.197	7.270
Cyrano B	104.333	1.333	122.582	1.943	252.530	3.308
Cyrano C	94.500	3.279	112.241	3.017	238.125	5.577
Cyrano D	96.000	3.606	110.450	3.539	216.235	6.174
Cyrano E	101.000	1.000	116.655	2.796	226.679	5.989
Cyrano F	99.533	2.149	116.657	2.383	225.945	4.136
Cyrano G	103.000	3.464	117.505	2.435	223.883	2.688
Cyrano H	92.533	2.598	107.627	2.166	220.220	5.948
Cyrano I	98.000	1.000	116.485	3.938	224.901	6.222
Cyrano J	93.067	1.348	105.722	1.413	221.522	2.443
Marathon A	86.667	1.333	107.688	2.178	231.117	6.197
Marathon B	94.000	1.732	110.685	1.624	231.694	5.608
Marathon C	77.450	2.599	91.869	3.772	221.661	10.104
Matador A	81.133	0.867	95.201	0.792	230.618	1.253
Matador B	58.333	5.783	98.919	1.223	213.875	2.983
Matador C	86.000	1.000	102.299	0.606	222.922	2.454
Matador D	108.000	2.000	124.425	2.411	246.504	6.411
Rizor A	90.000	3.819	101.280	4.200	202.320	7.678
Rizor B	78.167	14.412	93.537	6.862	191.907	8.902
Rizor C	81.333	2.333	89.587	2.563	189.160	8.191
Rizor D	73.100	13.109	87.020	4.814	196.840	7.565
Rizor E	94.667	4.910	102.480	6.380	205.847	10.864
Rizor F	95.833	4.206	106.413	4.815	213.017	7.493
Rizor G	71.667	13.220	88.260	5.210	189.173	9.471
Rizor H	80.333	15.496	96.303	9.686	201.050	11.633
Rizor I	90.167	5.890	97.523	5.494	197.760	4.036
Rizor J	92.667	6.360	100.363	4.924	208.803	5.460

Lot	standard test at 20°C									
	G% day 3		G% day 6		G% day 14		H% day 6		H% day 14	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano A	56.900	7.573	84.600	2.344	87.300	0.889	58.167	5.167	81.933	1.213
Cyrano B	61.867	10.196	83.633	2.835	85.300	4.158	54.567	13.706	83.967	3.983
Cyrano C	55.100	13.552	83.600	2.227	84.600	1.815	56.200	6.354	80.600	2.663
Cyrano D	49.000	11.590	81.667	1.453	82.667	1.453	58.667	11.921	79.000	1.528
Cyrano E	37.000	12.000	80.667	3.180	81.333	3.283	37.333	13.482	80.000	4.163
Cyrano F	43.333	12.197	82.333	0.667	82.000	0.577	41.333	15.667	79.333	0.882
Cyrano G	79.333	1.453	83.333	1.453	84.667	1.453	74.667	1.764	82.333	1.202
Cyrano H	88.000	2.646	91.000	2.082	91.000	2.082	82.667	4.842	89.000	3.215
Cyrano I	53.667	9.939	76.667	5.783	77.667	5.548	57.333	9.838	75.333	5.608
Cyrano J	82.667	9.871	92.333	2.028	93.000	1.732	81.333	6.888	91.000	3.215
Marathon A	76.000	9.074	98.333	0.667	98.667	0.882	71.333	10.171	97.333	0.882
Marathon B	67.767	18.315	96.000	0.577	97.333	0.882	54.000	24.637	96.000	0.577
Marathon C	92.333	4.177	98.333	1.667	98.333	1.667	76.333	8.373	96.667	2.028
Matador A	89.000	7.024	99.000	0.577	99.000	0.577	74.333	12.667	95.333	1.202
Matador B	94.667	0.667	97.000	0.577	97.000	0.577	78.333	4.807	93.667	0.667
Matador C	79.333	13.860	98.333	0.882	97.667	1.202	71.333	13.195	95.333	0.882
Matador D	54.667	18.496	96.000	0.577	97.000	1.000	49.333	23.024	96.667	1.333
Rizor A	52.633	12.846	87.933	2.621	88.633	2.338	49.567	24.351	86.967	2.090
Rizor B	87.667	1.856	94.000	2.082	95.000	1.528	83.333	8.413	93.333	1.333
Rizor C	58.267	26.056	92.267	3.908	93.330	3.286	59.000	22.605	90.933	4.107
Rizor D	48.333	16.374	95.333	1.667	95.667	1.856	47.333	22.908	93.667	1.453
Rizor E	84.000	3.464	95.333	0.333	96.000	0.000	73.667	13.220	95.000	0.577
Rizor F	54.500	25.257	91.467	0.837	93.167	0.590	60.967	22.993	92.467	0.731
Rizor G	64.667	20.407	95.000	2.517	95.333	2.728	53.000	26.230	94.667	2.848
Rizor H	75.000	4.583	93.667	0.667	94.333	0.667	64.000	12.662	92.667	0.667
Rizor I	54.333	19.650	92.667	0.882	93.667	0.882	51.333	20.755	92.000	0.577
Rizor J	74.433	4.158	93.300	0.850	95.333	1.856	77.933	6.871	94.333	1.453

Lot	cold sand test			
	sand 1-germination% day 14		sand 2-germination% day 14	
	mean	S.E.	mean	S.E.
Cyrano A	76.000	1.155	75.000	2.082
Cyrano B	83.000	1.000	83.000	1.000
Cyrano C	82.333	0.333	82.333	0.333
Cyrano D	78.333	2.333	78.333	2.333
Cyrano E	79.000	3.786	78.667	3.844
Cyrano F	80.000	1.528	80.000	1.528
Cyrano G	76.000	1.000	76.000	1.000
Cyrano H	80.000	1.528	80.000	1.528
Cyrano I	74.000	0.577	74.000	0.577
Cyrano J	77.333	3.180	76.333	3.283
Marathon A	91.667	2.333	91.667	2.333
Marathon B	93.000	2.082	92.333	2.333
Marathon C	95.333	1.333	93.333	1.764
Matador A	88.333	1.764	88.000	1.732
Matador B	89.467	2.054	85.667	4.842
Matador C	92.000	1.155	92.000	1.155
Matador D	93.333	0.882	93.333	0.882
Rizor A	92.000	2.082	91.333	1.764
Rizor B	93.333	2.333	91.000	2.517
Rizor C	86.667	2.728	85.333	3.528
Rizor D	94.667	0.882	93.000	1.155
Rizor E	97.000	1.528	94.667	2.333
Rizor F	93.333	2.404	91.333	2.333
Rizor G	97.333	1.453	95.667	1.202
Rizor H	93.667	1.202	91.333	1.856
Rizor I	93.000	1.528	92.333	2.186
Rizor J	93.000	0.577	91.667	0.882

Lot	wet stress test											
	G% day 4[1]		G% day 7		G% day 14		H% day 4[1]		H% day 7		H% day 14	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano A	72.900	0.100	76.567	2.248	80.267	2.876	29.400	3.113	71.900	1.626	79.933	3.034
Cyrano B	70.333	2.028	80.667	1.667	83.000	3.055	15.333	1.453	75.000	1.000	81.000	4.163
Cyrano C	79.000	2.646	81.667	1.667	84.000	1.000	23.000	4.359	75.333	1.667	83.667	1.202
Cyrano D	82.900	2.608	86.567	1.488	89.300	0.889	39.967	4.247	84.567	2.179	88.933	0.606
Cyrano E	72.500	4.851	77.200	4.743	78.867	3.152	34.233	3.580	71.867	4.967	77.200	3.113
Cyrano F	72.333	2.906	76.667	5.044	81.667	0.667	28.000	5.686	73.000	4.041	80.667	0.667
Cyrano G	71.333	2.728	75.667	2.028	80.000	1.000	36.000	2.646	68.667	3.480	78.000	1.000
Cyrano H	78.267	1.392	81.600	2.344	84.933	0.636	40.500	5.204	76.933	2.888	83.600	0.945
Cyrano I	70.667	0.333	75.000	0.577	79.333	2.186	19.667	2.906	71.333	0.882	78.333	2.667
Cyrano J	76.600	1.137	78.267	1.157	81.600	0.400	32.133	5.262	75.933	0.521	80.267	1.267
Marathon A	89.000	2.000	90.667	2.404	90.333	2.333	38.667	4.410	86.000	2.082	87.333	1.453
Marathon B	89.000	2.646	90.333	3.180	95.667	1.453	32.000	3.608	87.333	4.702	94.333	1.667
Marathon C	95.667	0.882	97.000	0.577	97.333	0.333	46.667	4.485	93.667	0.882	95.667	0.882
Matador A	88.667	5.897	89.667	2.848	92.000	2.309	42.333	6.642	83.000	6.110	89.667	2.963
Matador B	87.000	2.000	91.333	1.202	93.667	0.333	29.333	7.126	83.667	2.333	91.667	1.333
Matador C	91.000	1.732	92.333	2.603	95.000	2.082	45.000	2.517	89.667	3.756	94.667	2.404
Matador D	91.667	1.453	93.667	0.882	95.667	0.882	30.667	6.360	90.333	0.882	95.000	0.577
Rizor A	78.000	4.726	86.333	2.906	87.667	0.453	1.333	0.667	82.333	4.096	86.667	2.728
Rizor B	77.000	4.509	87.333	0.882	88.000	0.155	3.000	0.000	83.000	3.215	87.667	1.202
Rizor C	77.600	2.023	86.633	1.835	86.967	0.506	2.673	1.200	83.267	1.157	85.967	1.984
Rizor D	74.333	3.844	91.667	1.764	95.000	0.732	2.667	1.764	85.000	2.646	95.000	1.732
Rizor E	87.667	1.202	95.667	0.333	96.333	0.333	0.667	0.333	92.667	0.333	96.000	0.577
Rizor F	73.000	7.638	88.667	0.667	92.000	1.155	0.000	0.000	82.333	4.177	91.667	1.202
Rizor G	73.833	7.830	91.933	0.549	93.267	1.170	5.067	5.067	83.900	3.236	90.967	1.495
Rizor H	79.267	4.397	88.633	2.025	91.300	1.650	1.683	1.683	84.933	2.659	90.633	1.317
Rizor I	72.333	5.207	88.667	1.453	89.667	0.333	0.333	0.333	80.333	5.783	89.000	0.577
Rizor J	80.000	4.041	90.000	0.000	90.000	0.577	1.000	1.000	85.000	2.082	89.000	1.155

[1] day 3 for the cv. Rizor seedlots

APPENDIX C2 The germination at 9°C and 20°C of cv. Cyrano seedlots which were thiram-steeped or given an advancement treatment (mean of three replicates).

9°C	Seed treatment	G%		MGT (hours)		MHT (hours)	
Lot		mean	S.E.	mean	S.E.	mean	S.E.
B	steeped	72.333	1.856	214.747	2.972	478.317	12.862
G	steeped	73.667	2.186	227.700	2.984	489.960	21.785
H	steeped	84.000	2.082	186.660	5.365	440.250	5.533
B	advanced	82.333	1.764	128.423	11.459	302.243	13.349
G	advanced	88.000	1.528	107.877	4.651	270.303	6.737
H	advanced	86.967	1.539	98.803	3.042	271.773	9.999

20°C	Seed treatment	G%-day 3		G%-day 6		G%-day 14	
Lot		mean	S.E.	mean	S.E.	mean	S.E.
B	steeped	61.867	10.196	83.633	2.835	85.300	4.158
G	steeped	79.333	1.453	83.333	1.453	84.667	1.453
H	steeped	88.000	2.646	91.000	2.082	91.000	2.082
B	advanced	75.667	5.364	88.333	1.202	89.000	0.577
G	advanced	76.667	2.728	79.667	2.728	80.000	2.646
H	advanced	78.900	5.024	92.633	0.913	92.633	0.913

APPENDIX C3 The germination test results of seedlots 2 experiment (mean of three replicates).

Lot	cold stress test									
	T_{50} (days)		T_{30} (days)		T_{50}* (days)		MGT (days)		MHT (days)	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano G	8.867	0.394	8.183	0.297	8.483	0.366	9.191	0.379	19.736	0.828
Cyrano H	9.350	0.425	8.550	0.235	8.933	0.371	9.651	0.325	20.030	0.140
Rizor B	7.633	0.273	6.467	0.272	7.283	0.274	7.997	0.402	19.895	0.806
Rizor F	9.033	0.606	8.300	0.414	8.717	0.536	9.622	0.584	19.653	0.471
Matador B	9.033	0.623	7.933	0.471	8.700	0.550	9.529	0.723	22.025	1.396
Matador D	9.450	0.104	8.867	0.131	9.367	0.186	10.314	0.221	21.360	0.599

Lot	standard test at 20°C											
	G% day 4		G% day 7		G% day 15		H% day 4		H% day 7		H% day 15	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano G	77.333	0.667	78.000	0.000	78.933	0.581	3.000	0.577	76.667	0.667	77.600	0.400
Cyrano H	85.000	3.055	85.333	2.333	86.000	2.309	8.667	4.256	83.000	2.646	84.333	2.906
Rizor B	92.667	0.667	95.000	0.577	95.000	0.577	15.000	4.041	93.333	1.202	93.667	0.882
Rizor F	93.667	0.333	94.667	0.333	95.000	0.577	2.000	1.000	92.333	0.333	94.667	0.882
Matador B	94.667	0.333	95.000	0.577	95.000	0.577	2.333	1.453	90.667	0.333	91.333	0.333
Matador D	97.000	1.528	99.000	0.577	99.000	0.577	0.667	0.667	97.333	1.202	98.333	0.333

Lot	cold sand test	
	1-G% day 14	
	mean	S.E.
Cyrano G	75.667	0.882
Cyrano H	77.667	2.603
Rizor B	94.000	0.577
Rizor F	90.000	2.082
Matador D	90.333	4.096
Matador B	78.667	2.333

Lot	wet stress test													
	G% day 2		G% day 4		G% day 7		G% day 14		H% day 4		H% day 7		H% day 14	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano G	58.067	0.636	72.137	1.442	73.137	1.985	73.817	1.597	34.947	4.316	71.470	1.811	73.483	1.807
Cyrano H	48.667	1.453	80.667	3.712	82.333	4.333	82.333	3.333	31.667	2.333	79.667	4.177	80.667	2.667
Rizor B	69.667	5.239	92.333	0.882	93.000	0.577	94.333	1.333	51.000	2.646	92.667	0.667	93.667	0.667
Rizor F	65.000	1.000	90.333	2.848	92.667	2.333	93.333	2.333	31.000	2.309	89.333	3.528	92.000	3.606
Matador B	60.000	6.658	88.000	2.309	91.000	1.528	90.333	0.333	23.667	6.009	86.667	2.404	87.000	1.528
Matador D	40.667	2.906	93.667	1.202	95.000	0.577	95.333	1.202	8.667	1.202	90.000	1.528	93.667	1.856

APPENDIX C4 The germination of treated cv. Cyrano H seeds in the standard test at 20°C (mean of three replicates).

Seed treatment	G%-day 4		G%-day 7		G%-day 15	
	mean	S.E.	mean	S.E.	mean	S.E.
untreated	59.000	10.817	81.000	1.528	83.667	2.333
aged	43.333	4.333	71.333	3.180	75.467[1]	2.533
advanced	92.300	0.907	92.633	0.913	92.967[2]	1.033
advanced/aged	76.333	1.333	78.667	0.882	85.333[3]	1.453

the percentage of abnormals: [1]=1.43%, [2]=0.33%, [3]=9.67%

APPENDIX D1 The RNA/DNA ratios of seedlots 1 (mean of three replicates except bold indicates mean of four replicates).

Seedlot	RNA/DNA ratios					
	1		2		3	
	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano A	3.685	0.227	4.101	0.230	1.781	0.146
Cyrano B	3.766	0.327	4.144	0.328	1.544	0.116
Cyrano C	3.904	0.244	4.296	0.236	1.700	0.101
Cyrano D	3.854	0.050	4.242	0.071	1.816	0.026
Cyrano E	3.824	0.190	4.260	0.198	1.795	0.141
Cyrano F	3.915	0.345	4.353	0.370	1.845	0.208
Cyrano G	3.378	0.183	3.729	0.238	1.514	0.086
Cyrano H	4.087	0.249	4.530	0.267	1.814	0.212
Cyrano I	3.912	0.258	4.346	0.268	1.780	0.185
Cyrano J	3.636	0.035	3.911	0.081	1.554	0.099
Marathon A	4.419	0.401	4.875	0.462	2.329	0.334
Marathon B	4.374	0.192	4.877	0.211	2.282	0.175
Marathon C	4.327	0.060	4.824	0.059	2.200	0.021
Matador A	4.244	0.632	4.708	0.721	2.168	0.393
Matador B	4.748	0.312	5.319	0.354	2.520	0.159
Matador C	4.240	0.201	4.731	0.229	2.138	0.171
Matador D	3.737	0.448	4.133	0.512	1.849	0.313
Rizor A	**6.006**	0.266	**6.538**	0.243	**3.028**	0.054
Rizor B	6.839	0.712	7.669	0.822	3.513	0.339
Rizor C	**6.268**	0.116	**6.968**	0.133	**3.324**	0.108
Rizor D	**5.560**	0.123	**6.162**	0.133	**2.892**	0.091
Rizor E	**6.189**	0.207	**6.929**	0.226	**3.222**	0.086
Rizor F	**6.063**	0.466	**6.708**	0.491	**3.013**	0.107
Rizor G	**5.985**	0.186	**6.655**	0.222	**3.104**	0.114
Rizor H	**5.981**	0.079	**6.665**	0.108	**3.222**	0.092
Rizor I	**6.068**	0.242	**6.775**	0.275	**3.168**	0.136
Rizor J	**6.017**	0.243	**6.710**	0.262	**3.118**	0.086

APPENDIX D2 The RNA/DNA ratios of thiram-steeped and advanced cv. Cyrano seedlots (mean of four replicates).

Lot	Seed treatment	RNA/DNA ratios							
		1		2		3		4	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
B	steeped	4.431	0.273	4.891	0.298	1.940	0.172	4.230	0.250
G	steeped	5.170	0.225	5.790	0.252	2.680	0.127	5.051	0.220
H	steeped	5.399	0.522	6.057	0.598	2.722	0.223	5.263	0.507
B	advanced	6.585	0.684	7.420	0.781	3.378	0.350	6.431	0.655
G	advanced	6.897	1.259	7.820	1.455	3.742	0.696	6.796	1.242
H	advanced	6.563	0.706	7.446	0.810	3.534	0.370	6.455	0.699

APPENDIX D3 The RNA/DNA ratios of selected seedlots (seedlots 2 experiment) of cvs. Cyrano, Rizor and Matador (mean of six replicates).

Lot	RNA/DNA ratios																			
	1		2		3		4		5		6		7		8		9		10	
	mean	S.E	mean	S.E	mean	S.E	mean	S.E	mean	S.E	mean	S.E	mean	S.E	mean	S.E	mean	S.E	mean	S.E
Cyrano G	5.297	0.201	5.810	0.224	2.324	0.138	5.059	0.184	12.520	0.526	10.016	0.421	5.930	0.342	4.744	0.274	11.995	0.504	9.596	0.403
Cyrano H	5.664	0.405	6.259	0.455	2.650	0.206	5.462	0.440	13.555	0.962	10.844	0.770	6.771	0.499	5.416	0.399	13.106	1.046	10.484	0.836
Rizor B	6.101	0.502	6.600	0.520	2.445	0.075	5.649	0.467	15.270	1.281	12.216	1.025	6.643	0.243	5.314	0.195	14.212	1.208	11.369	0.966
Rizor F	6.712	1.185	7.208	1.217	2.399	0.204	6.237	1.110	17.073	2.915	13.658	2.332	6.701	0.608	5.361	0.486	15.912	2.708	12.730	2.167
Matador B	5.448	0.353	5.990	0.398	2.509	0.169	5.174	0.369	12.398	0.824	9.918	0.659	6.090	0.383	4.872	0.307	11.808	0.848	9.447	0.679
Matador D	5.259	0.432	5.737	0.477	2.214	0.176	4.969	0.435	12.224	0.852	9.779	0.681	5.583	0.347	4.466	0.277	11.586	0.844	9.268	0.675

APPENDIX D4 Summary of RNA/DNA ratios of size-graded fruits (mean of six replicates except bold indicates mean of five replicates). Fruit sizes are: 1 (<3.5 mm in diameter), 3 (3.75-4 mm), 5 (4.25-4.5 mm), 7 (4.75-5 mm), 9 (5.25-5.5 mm) and 10 (>5.5 mm).

Size	RNA/DNA ratios							
	1		2		3		4	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
1	4.108	0.405	4.555	0.464	2.024	0.265	3.973	0.400
3	**4.126**	0.641	**4.545**	0.723	**1.979**	0.380	**3.892**	0.597
5	4.188	0.609	4.673	0.672	2.035	0.429	4.032	0.554
7	4.787	0.561	5.356	0.649	2.404	0.387	4.660	0.545
9	4.570	0.301	5.066	0.341	2.205	0.244	4.406	0.293
10	4.703	0.503	5.247	0.583	2.244	0.400	4.563	0.489

APPENDIX D5 The concentrations (in μg/ml) of nucleic acids dissolved in solution extracted from the true seeds of size-graded fruits.

Size	DNA	RNA (number corresponds to the RNA/DNA calculation)			
		1	2	3	4
1	140.32	589.85	654.68	292.35	570.67
3	155.92	673.72	743.10	326.92	635.49
5	159.03	715.63	799.47	358.97	688.77
7	180.95	909.92	1020.06	465.20	886.12
9	210.00	992.42	1102.21	489.90	958.35
10	184.17	909.73	1016.61	449.03	883.45

APPENDIX D6 The RNA/DNA ratios of diploid and triploid varieties (mean of three replicates).

Seed bulk	Ploidy	Pair	RNA/DNA ratios							
			1		2		3		4	
			mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Regina	triploid	1	6.265	0.049	7.104	0.070	3.417	0.039	6.105	0.059
Roberta	diploid	1	5.704	0.272	6.394	0.300	2.998	0.162	5.496	0.282
Zulu	triploid	2	5.769	0.249	6.501	0.291	3.072	0.130	5.565	0.224
Saxon	diploid	2	5.882	0.321	6.636	0.362	3.263	0.201	5.698	0.322
Rose	triploid	3	6.323	0.257	7.131	0.311	3.343	0.158	6.108	0.260
Druid	diploid	3	6.491	0.170	7.344	0.151	3.554	0.114	6.305	0.129
Hilma	triploid	4	6.469	0.228	7.312	0.255	3.538	0.079	6.230	0.198
Rex	triploid	4	6.264	0.009	7.058	0.011	3.510	0.064	6.037	0.025

APPENDIX D7 The concentrations of nucleic acids (μg/ml) dissolved in solution extracted from the true seeds of cultivars which are diploid or triploid.

Seed bulk	Ploidy	Pair	DNA	RNA (number refers to RNA/DNA calculation)			
				1	2	3	4
Regina	triploid	1	149.07	934.07	1059.30	509.07	910.27
Roberta	diploid	1	131.47	752.99	844.06	395.99	725.79
Zulu	triploid	2	132.46	766.47	863.67	408.79	739.74
Saxon	diploid	2	121.96	727.94	821.21	404.27	705.51
Rose	triploid	3	159.43	1009.41	1138.22	534.09	975.01
Druid	diploid	3	143.43	929.73	1052.25	508.81	903.21
Hilma	triploid	4	158.30	1021.88	1155.09	559.48	984.48
Rex	triploid	4	141.70	887.60	1000.24	496.60	855.64

APPENDIX D8 The RNA/DNA ratios of treated seedlots of cv. Cyrano (mean of four replicates).

Lot	Seed treatment	RNA/DNA ratios							
		1		2		3		4	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
B	steeped	4.431	0.273	4.891	0.298	1.940	0.172	4.230	0.250
G	steeped	5.170	0.225	5.790	0.252	2.680	0.127	5.051	0.220
H	steeped	5.399	0.522	6.057	0.598	2.722	0.223	5.263	0.507
B	advanced	6.585	0.684	7.420	0.781	3.378	0.350	6.431	0.655
G	advanced	6.897	1.259	7.820	1.455	3.742	0.696	6.796	1.242
H	advanced	6.563	0.706	7.446	0.810	3.534	0.370	6.455	0.699
B	untreated	4.965	0.888	5.571	1.033	2.274	0.627	6.431	0.655
G	untreated	5.459	0.432	6.130	0.485	2.803	0.154	4.735	0.878
H	untreated	5.666	1.049	6.354	1.179	2.735	0.391	5.283	0.447

APPENDIX D9 The concentrations of nucleic acids (µg/ml) dissolved in solution extracted from the true seeds of treated seedlots of cv. Cyrano.

Lot	Seed treatment	DNA	RNA (number refers to RNA/DNA calculation)			
			1	2	3	4
B	steeped	167.50	740.14	817.07	325.00	706.99
G	steeped	187.23	965.19	1081.03	501.60	942.60
H	steeped	189.78	1004.35	1126.16	508.12	979.06
B	advanced	226.05	1486.35	1675.90	762.15	1452.18
G	advanced	238.51	1726.79	1960.35	938.84	1702.31
H	advanced	226.80	1478.10	1676.43	796.23	1453.62
B	untreated	149.55	763.17	856.78	353.64	729.51
G	untreated	175.25	971.60	1091.10	497.81	942.02
H	untreated	164.25	928.38	1041.42	448.98	898.80

APPENDIX D10 The RNA/DNA ratios of treated cv. Cyrano H seeds (mean of six replicates).

Seed treatment	RNA/DNA ratios							
	1		2		3		4	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
untreated	5.905	0.274	6.593	0.309	3.141	0.143	5.707	0.258
aged	6.286	0.146	7.027	0.158	3.321	0.080	6.032	0.151
advanced	7.045	0.386	8.038	0.454	3.913	0.245	6.953	0.383
advanced/ aged	7.043	0.116	8.000	0.142	3.797	0.067	6.938	0.114

APPENDIX E1 The results of field trial 1 (mean of four replicates except bold indicates mean of three and italics mean of two replicates). For the results of ANOVA and levels of significance see section 3.2.1.

Lot	sum%		establishment%		MET (days)		T_{50} (days)		T_{50}* (days)		T_{30} (days)	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano A	57.000	1.013	55.300	0.957	25.836	0.618	29.375	1.028	23.375	0.239	23.500	0.354
Cyrano B	55.800	0.622	54.800	0.849	26.966	0.396	33.500	0.645	23.625	0.239	24.000	0.289
Cyrano C	53.400	2.754	52.400	2.658	27.106	0.523	**35.000**	5.508	23.625	0.515	24.750	0.829
Cyrano D	61.700	2.468	60.900	2.081	25.184	0.772	27.375	2.014	22.625	0.554	22.500	0.677
Cyrano E	53.700	3.022	52.100	2.918	25.674	1.059	**37.167**	9.968	22.750	0.250	23.375	0.375
Cyrano F	56.000	2.000	54.100	1.684	26.619	0.359	36.500	6.551	23.250	0.250	24.000	0.354
Cyrano G	66.000	2.059	64.600	1.865	24.397	0.705	24.500	0.456	21.625	0.688	21.500	0.612
Cyrano H	60.200	2.615	58.400	3.085	24.528	0.160	27.375	1.068	22.375	0.239	22.000	0.000
Cyrano I	50.500	3.828	49.500	3.971	26.432	0.935	*29.500*	1.500	23.125	0.427	24.125	0.966
Cyrano J	56.100	1.418	55.400	1.501	26.892	0.458	32.750	2.278	23.250	0.144	23.500	0.289
Marathon A	65.800	3.260	64.700	3.328	26.301	0.775	26.250	1.451	23.500	0.645	23.000	0.842
Marathon B	63.200	1.071	62.100	1.100	25.306	0.560	26.625	0.944	22.625	0.427	22.375	0.427
Marathon C	78.700	2.419	76.200	3.031	24.234	0.624	22.625	0.875	22.000	0.677	20.875	0.515
Matador A	72.000	1.966	69.100	1.473	23.814	0.678	23.250	0.722	21.125	0.657	20.625	0.657

Matador B	73.700	2.700	72.800	2.628	22.738	0.554	22.750	0.323	20.500	0.540	20.000	0.408
Matador C	70.400	3.051	68.200	2.553	24.897	0.737	24.250	0.854	22.250	0.595	22.000	0.707
Matador D	72.500	4.011	71.100	3.985	26.369	0.532	25.375	1.087	23.313	0.188	22.750	0.433

APPENDIX E2 The results of field trial 2 (means of four replicates except bold indicates mean of three replicates and italics mean of one replicate). For the results of ANOVA and levels of significance see section 3.2.2.

Lot	Seed treatment	sum%		establishment%		MET (days)		T_{50} (days)	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano B	steeped	45.700	2.615	44.700	2.584	28.282	0.834	*46.000*	
Cyrano B	advanced	69.400	2.517	67.800	2.312	18.037	0.201	21.000	0.408
Cyrano G	steeped	55.000	0.529	52.800	0.879	25.495	0.619	32.750	2.016
Cyrano G	advanced	68.800	0.589	67.600	0.589	15.597	0.179	18.250	0.250
Cyrano H	steeped	56.800	2.269	55.000	2.605	26.232	0.719	36.250	5.935
Cyrano H	advanced	65.000	1.545	63.200	1.523	15.913	0.369	19.625	0.239
Matador B	steeped	64.500	4.200	62.600	4.152	24.727	0.625	26.750	2.046
Matador B	advanced	**73.867**	2.243	**71.067**	1.764	**18.304**	0.377	**20.167**	0.167
Matador C	steeped	**63.200**	3.816	**62.267**	3.668	**26.593**	0.909	**30.000**	3.000
Matador C	advanced	79.400	1.390	77.800	1.915	15.827	0.398	18.000	0.289

Matador D	steeped	65.800	2.094	64.900	2.300	27.759	0.414	27.500	0.645
Matador D	advanced	88.300	2.187	86.000	2.953	16.156	0.250	16.625	0.125

APPENDIX E3 The results of the performance in field trial 3 of cv. Planet seeds which were rubbed and density-graded (mean of four replicates). For the results of ANOVA and levels of significance see section 3.2.3.1.

Rubbing	Density	establishment%		MET (days)		T_{50} (days)		T_{50}* (days)		T_{30} (days)	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
none	heavy	67.535	6.695	32.608	0.405	35.375	2.561	27.525	0.439	27.563	0.938
none	medium	58.810	3.483	32.260	0.500	37.875	1.712	27.275	0.634	27.938	1.371
light	heavy	71.163	3.345	30.998	0.493	31.063	1.847	26.400	0.220	25.875	0.415
light	medium	61.723	5.849	31.520	0.737	36.813	1.706	26.338	0.256	26.125	0.239
normal	heavy	72.915	1.505	31.110	0.285	31.250	1.127	26.363	0.152	25.625	0.125
normal	medium	61.200	1.826	30.215	0.546	34.375	2.055	25.750	0.144	25.563	0.258
heavy	heavy	82.510	3.633	30.338	0.443	27.375	0.554	25.825	0.165	24.625	0.239
heavy	medium	64.270	5.011	29.975	0.886	31.750	3.301	25.788	0.539	25.375	0.591

APPENDIX E4 The results of the performance in field trial 3 of cvs. Rizor and Cyrano seedlots (mean of four replicates except bold indicates mean of three replicates and italic indicates mean of two replicates). For the results of ANOVA and levels of significance see section 3.2.3.2.

Lot	establishment%		MET (days)		T_{50} (days)		T_{50}* (days)		T_{30} (days)	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Rizor A	59.385	3.044	31.520	0.539	37.000	1.568	26.613	0.153	26.625	0.161
Rizor B	61.700	3.642	29.448	0.373	33.500	2.661	25.438	0.329	25.375	0.515
Rizor C	60.153	1.873	29.598	0.613	34.625	2.750	24.875	0.298	24.875	0.427
Rizor D	64.235	4.571	31.003	0.953	34.625	2.609	26.875	0.554	26.125	0.554
Rizor E	63.565	2.881	31.230	0.942	33.575	2.280	27.063	0.695	26.375	0.473
Rizor F	56.645	5.371	30.210	0.826	**34.167**	4.475	25.400	0.501	25.625	0.554
Rizor G	61.378	3.704	31.393	0.709	35.250	2.487	26.075	0.048	27.000	1.021
Rizor H	63.770	4.094	30.813	1.074	34.563	2.773	26.475	0.910	25.875	0.681
Rizor I	61.203	5.144	30.508	0.998	35.000	3.342	25.800	0.549	26.813	1.847
Rizor J	65.600	1.932	28.518	0.502	28.563	1.821	25.375	0.290	25.000	0.408
Cyrano B	50.200	2.425	29.505	1.075	*36.500*	1.500	25.175	0.236	28.125	2.313
Cyrano G	53.413	3.079	29.238	1.126	**33.833**	3.898	24.688	0.547	26.500	1.893
Cyrano H	53.115	2.049	29.085	0.386	38.750	2.287	24.900	0.311	25.500	0.289

APPENDIX E5 A summary of the field performance of cv. Cyrano B, G and H seedlots in trial 1, 2 and 3 (mean of four replicates except bold indicates mean of one replicate, italic indicates mean of two and underlined indicates mean of three replicates).

Lot	establishment%			MET (days)			T_{50} (days)			T_{50}* (days)			T_{30} (days)		
	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
B	54.800	44.700	50.200	26.966	28.282	29.505	33.500	**46.000**	*36.500*	23.625	23.750	25.175	24.000	27.125	28.125
G	64.600	52.800	53.413	24.397	25.495	29.238	24.500	32.750	33.833	21.625	22.625	24.688	21.500	23.125	26.500
H	58.400	55.000	53.115	24.528	26.232	29.085	27.375	36.250	38.750	22.375	22.500	24.900	22.000	22.750	25.500

APPENDIX E6 The performance of seedlots 2 in the field (mean of eight replicates). For the results of ANOVA and levels of significance see section 3.2.4.

Lot	sum%		establishment%		MET (days)		T_{30} (days)		T_{50}* (days)	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano G	53.833	2.970	50.000	3.304	22.559	0.515	21.344	0.716	20.188	0.235
Cyrano H	49.917	1.439	45.083	1.297	22.509	0.350	21.563	0.258	20.625	0.142
Rizor B	58.417	2.134	51.750	2.218	22.131	0.386	20.219	0.297	19.813	0.181
Rizor F	61.750	1.175	55.750	0.925	22.669	0.236	20.938	0.103	20.625	0.134
Matador B	57.833	2.159	52.417	1.890	23.411	0.343	21.094	0.200	20.875	0.254
Matador D	64.417	2.979	58.500	2.924	23.371	0.424	21.094	0.372	20.844	0.163

APPENDIX E7 The fresh weights (g) of harvested seedlings of selected seedlots (seedlots 2 experiment) from cvs. Cyrano, Rizor and Matador (a = emerged early, b =emerged in the middle and c = emerged late in the counting period). For the results of ANOVA and levels of significance see section 3.2.4.

Lot	fresh weight a (g)		fresh weight b (g)		fresh weight c (g)		total fresh weight (g)	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano G	56.025	11.762	108.493	17.470	13.653	1.425	178.170	20.955
Cyrano H	42.506	5.783	102.221	6.477	13.055	1.519	157.783	8.309
Rizor B	73.841	8.938	67.631	10.147	21.866	11.294	163.339	11.659
Rizor F	36.980	3.886	130.224	8.696	18.085	1.153	185.289	10.510
Matador B	31.740	7.275	124.656	10.949	17.349	1.938	173.745	15.530
Matador D	31.683	8.210	163.775	15.037	23.470	1.365	218.928	20.937

Lot	fresh weight/plant a (g)		fresh weight/plant b (g)		fresh weight/plant c (g)		total fresh weight/plant (g)	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano G	3.401	0.158	2.400	0.190	1.066	0.140	2.352	0.202
Cyrano H	3.370	0.129	2.370	0.124	1.204	0.133	2.337	0.111
Rizor B	2.831	0.104	1.746	0.245	2.307	1.469	2.092	0.084
Rizor F	2.970	0.195	2.322	0.120	1.213	0.090	2.212	0.112
Matador B	3.048	0.229	2.461	0.115	0.938	0.074	2.183	0.118
Matador D	3.277	0.490	2.731	0.138	1.207	0.102	2.463	0.163

APPENDIX E8 The dry weights (g) of harvested seedlings of selected seedlots (seedlots 2 experiment) from cvs. Cyrano, Rizor and Matador (a = emerged early, b = emerged in the middle and c = emerged late in the counting period). For the results of ANOVA and levels of significance see section 3.2.4.

Lot	dry weight a (g)		dry weight b (g)		dry weight c (g)		total dry weight (g)	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano G	5.419	1.203	10.353	1.601	1.316	0.134	17.088	2.009
Cyrano H	4.008	0.537	9.640	0.577	1.226	0.155	14.874	0.751
Rizor B	7.338	0.939	7.470	0.521	2.191	1.124	17.049	1.524
Rizor F	3.630	0.378	12.631	0.837	1.830	0.122	18.091	1.027
Matador B	3.130	0.722	11.975	0.859	1.779	0.196	16.884	1.369
Matador D	3.014	0.784	15.304	1.328	2.284	0.138	20.601	1.908

Lot	dry weight/plant a (g)		dry weight/plant b (g)		dry weight/plant c (g)		total dry weight/plant (g)	
	mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
Cyrano G	0.321	0.009	0.229	0.016	0.102	0.012	0.224	0.018
Cyrano H	0.318	0.013	0.224	0.011	0.111	0.011	0.221	0.011
Rizor B	0.282	0.011	0.191	0.012	0.231	0.146	0.218	0.014
Rizor F	0.292	0.019	0.225	0.012	0.123	0.009	0.216	0.011
Matador B	0.302	0.024	0.238	0.009	0.096	0.008	0.213	0.011
Matador D	0.313	0.047	0.256	0.012	0.117	0.010	0.232	0.015